Techno-economic analysis of the valorisation of brewers spent grains: Production of xylitol and xylo-oligosaccharides

by

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ABSTRACT

Techno-economic analysis of the valorisation of brewers spent grains: Production of xylitol and xylo-oligosaccharides

Brewers' spent grains (BSG) make up to 85% of breweries' solid waste produced, and is either sent to landfill or sold as cheap animal feed supplement. Yet a valuable sugar replacer, xylitol, and/or prebiotic xylo-oligosaccharides (XOS) can be produced from BSG, which can be used as supplements or in food formulations as low calories sweeteners and texturisers. Both are high value commodities (>US$5/kg) with a projected annual market growth greater than 10%.

Hydrothermal fractionation technologies, Liquid Hot Water (LHW) and Steam Explosion (SE) have been reported for extracting a fraction of XOS and xylose (for xylitol production) from BSG. However, the reported autocatalytic LHW processes for BSG use high temperatures (>180 °C) and in low dry matter (dm) concentrations (<11% dm), while no such SE processes have been reported. Therefore, the intensification of hydrothermal treatment (HTT) processes for BSG was investigated, through reductions in both the required temperatures and process water usage, to improve process economics, through screw press dewatering of BSG prior to HTT treatment.

Firstly, HTT process intensification for production of XOS was demonstrated in a 1 L LHW batch reactor with a Weiss BSG, raw (15% dm) and screw pressed (25% dm), combined with extremely low acid (ELA) catalyst dosing (5, 12.5 and 20 mg H_2SO_4/g dry mass). Improved XOS yields (61.4–76.4%) were obtained using >15% dm under reduced temperature conditions (150–170 °C), compared to previous reports with 9–11% dm. Screw pressing BSG enhanced the ELA acidification in LHW HTT with up to 400% more H^+ released through the selective removal of dissolved solids, ash, starches and proteins during the screw press dewatering.

Secondly, the preferred process conditions from LHW HTT was successfully scaled-up to a 19 L SE reactor, for both Weiss and Malt BSGs, achieving similar and higher XOS yields (>73%) by reducing the water content with a screw press by more than 60%. The preferred process conditions were insensitive to variations of BSG chemical compositions. SE severity adjustment through SO_2 catalyst addition could increase the fraction of valuable short chain oligomers (xylobiose and xylotriose) in the isolated XOS, from 7.5% to 25.3%.

Economic assessments were conducted from three simulations conducted in ASPEN Plus® of the preferred process configurations, aided with experimental data to improve accuracy of process models. All scenarios showed internal rates of return greater than the hurdle rate

(9.7%), at the assumed market price of US$4500/t for both xylitol and XOS. Yet XOS was more favourable with a minimum required selling price (MRSP) of US$2509/t, compared to the more elaborate production process for xylitol (MRSP of US$4153/t). By using an appropriate HTT, a scenario for the combined production of both xylitol and XOS could be defined that achieved the lowest MRSP of US$2182/t. By-products including BSG protein contributed 32.7%, 14.2% and 27.5% to the total process revenue for xylitol, XOS and the combination respectively. Finally, a small scale biorefinery annexed to a large brewery was shown to be viable combining high value products and the HTT process intensification achieved in this study.

OPSOMMING

Tegno-ekonomiese analises van die valorisasie van brouer gebruikte graan: Produksie van xilitol en xilo-oligosakkariedes

Brouer gebruikte graan (BSG) maak tot 85% van brouerye se geproduseerde soliede afval uit, en word óf na die vullisterrein gestuur, óf as goedkoop dierevoersupplement verkoop. ’n Waardevolle suikerplaasvervanger, xilitol, en/of prebiotiese xilo-oligosakkariede (XOS) kan vervaardig word uit BSG, wat gebruik kan word as supplemente of in voedselformulasies as lae kalorieë versoeters en teksturiseerders. Beide is hoë waarde kommoditeite (>US$5/kg) met ’n geprojekteerde jaarlikse markgroei groter as 10%.

Hidrotermiese fraksioneringstegnologieë, vloeistof warm water (LHW) en stoomontploffing (SE) is gerapporteer om ’n fraksie van XOS en xilose (vir xilitolproduksie) uit BSG te ekstraheer. Die gerapporteerde outokatalitiese LHW-prosesse vir BSG gebruik egter hoë temperature (>180 °C) en in lae droë stof (dm)-konsentrasies (<11% dm), terwyl geen sulke SE-prosesse gerapporteer is nie. Daarom is die intensifikasie van hidrotermiese behandeling (HTT)-prosesse vir BSG ondersoek, deur verminderings in beide die vereiste temperature en proses watergebruik, om prosesekonomië te verbeter, deur skroefpersontwatering van BSG voor HTT-behandeling.

Eerstens is HTT-prosesintensifikasie vir die vervaardiging van XOS gedemonstreer in ’n 1 L LHW-lotreaktor met ’n Weiss BSG, rou (15% dm) en skroefgepers (25% dm), gekombineer met ’n uiterse lae suur (ELA)-katalisdosering (5, 12.5 en 20 mg H_2SO_4/g droë massa). Verbeterde XOS-opbrengste (61.4–76.4%) is verkry deur >15% dm onder verlaagde temperatuurkondisies (150–170 °C), in vergelyking met vorige verslae met 9–11% dm. Deur BSG te skroefpers verbeter die ELA-versuring in LHW HTT met tot 400% meer H+ wat vrygestel word deur die selektiewe verwydering van opgeloste vaste stowwe, as, koolhidrate en proteïene tydens die skroefpersontwatering.

Tweedens is die gekose proseskondisies van LHW HTT suksesvol opgeskaal tot ’n 19 L SE-reaktor, vir beide Weiss- en Malt-BSGs, wat soortgelyke en hoë XOS-opbrengste (>73%) bereik het deur die waterinhoud met ’n skroefpers met meer as 60% te verminder. Die gekose proseskondisies was onsensitief vir variasies van BSG chemiese komposisies. SE-intensiteitwysiging deur byvoeging van SO_2-katalis kon die fraksie waardevolle kortkettingoligomere (xilobiose en xilotriose) in die geïsoleerde XOS verhoog, van 7.5% tot 25.3%.

Ekonomiese assesserings is gedoen uit drie simulasies uitgevoer in ASPEN Plus® van die gekose proseskonfigurasies, ondersteun deur eksperimentele data om die akkuraatheid van

die prosesmodelle te verbeter. Alle scenario's het interne opbrengskoerse groter as die versperringskoers (9.7%) getoon, by die aangenome markprys van US$4500/t vir beide xilitol en XOS. XOS was egter meer gunstig met 'n minimum vereiste verkoopsprys (MRSP) van US$2509/t, in vergelyking met die meer omslagtige vervaardigingsproses vir xilitol (MSRP van US$4153/t). Deur 'n gepaste HTT te gebruik, kon 'n scenario vir die gekombineerde vervaardiging van beide xilitol en XOS gedefinieer word wat die laagste MRSP van US$2182/t bereik het. By-produkte, wat BSG-proteïene insluit, het 32.7%, 14.2% en 27.5% tot die totale prosesinkomste vir xilitol, XOS en die kombinasie onderskeidelik bygedra. Laastens, 'n kleinskaalbioraffinadery langs 'n grootskaalse brouery is in hierdie studie bewys om lewensvatbaar te wees deur hoë volume produkte en die HTT-prosesintensifisering te kombineer.

Declaration of contributions

Declaration by the candidate:

With regard to [specify chapter or part of a chapter and page numbers in the dissertation], the nature and scope of my contribution were as follows:

Nature of contribution	Chapter	Extent of contribution (%)
Problem definition	1	100%
Experimental work (excluding HPLC)	4 and 5	100%
Aspen Model and simulations	6	100%
Thesis layout and write-up	1 to 7	95%

The following co-authors have contributed to:

Chapter	Name	Nature of contribution	Extent of contribution (%)
1,2 and 3,7	**OB** **AP** **EvR** **JG**	Internal reviewer, language Internal reviewer, language Internal reviewer, language Internal reviewer, language	**10** **10** **10** **10**
4	**OB** **AP** **EvR** **JG**	Internal reviewer, language Internal reviewer, language Internal reviewer, language Study leader, Research guidance, language	**10** **5** **10** **10**
5	**OB** **AP** **EvR** **JG**	Internal reviewer, language Internal reviewer, language Internal reviewer, language Study leader, Research guidance, language	**10** **5** **10** **10**
6	**OB** **AP** **EvR** **JG**	Internal reviewer, language Research guidance, language Internal reviewer, language Study leader, Research guidance, language	**10** **5** **5** **10**

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The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors,

2. no other authors contributed to the dissertation besides those specified above, and

3. no potential conflicts of interest to all interested parties exist in this dissertation.

Signature Institutional affiliation Date

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This work I dedicate to my dearest mother Shana

Prof Johann Görgens for pushing me to do “a handful” of experiments for my techno-economic analysis

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“Preoccupation with efficacy is the main obstacle to a poetic, elegant, robust and heroic life.”

(Taleb, 2010)

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Abbreviations and nomenclature

%ArOS	percentage arabino-oligomers of total arabinan yield
%GlcOS	percentage gluco-oligomers of total glucan yield
%XOS	percentage xylo-oligomers of total xylan yield
°C	degrees Celsius
ANOVA	analysis of variance
ASTM	American Society for Testing and Materials
ArOS	arabino-oligosaccharide
AXOS	arabinoxylo-oligosaccharide
BCS	brewer's condensed solubles
BSG	brewer's spent grains
BDG	brewer's dried grains
BDP	brewer's dried proteins
BPG	brewer's pressed grains
ca	*circa* 'approximately'
C5	five carbon
C6	six carbon
CAPEX	capital expense
CAGR	compound annual growth rate
CSF	combined severity function
DA	dilute acid
dm	dry matter
DP	degree of polymerisation
DS	dissolved solids
ELA	extremely low acid
FCI	fixed capital investment
FOS	fructo-oligosaccharide
GlcOS	gluco-oligosaccharide
GOS	galacto-oligosaccharide
H+	hydrogen ion
HMF	5-Hydroxymethyl furfural
HPLC	high performance liquid chromatography
IRR	internal rate of return
IS	insoluble solids
ISBL	inside battery limits (of the plant)
kDa	kilo Dalton
LAP	Laboratory Analytical Procedure
LC	lignocellulose
LCM	lignocellulosic material
LHW	liquid hot water
LSR	liquid solid ratio
mg	milligram
min	minutes

MM	million
MRSP	minimum required selling price
MWCO	molecular weight cut off
NDF	neutral detergent fibre
NPV	nett present value
NREL	National Research Energy Laboratory
OPEX	operating expense
OS	oligosaccharide
PBP	payback period
PBSG-15%	15% dry matter Pale malt brewer's spent grains
PBSG-25%	25% dry matter Pale malt brewer's spent grains
PBSG-32%	32% dry matter Pale malt brewer's spent grains
PFD	process flow diagram
SD	standard deviation
SE	steam explosion
t	metric tonne
T	temperature
TAPPI	Technical Association of the Pulp and Paper Industry
TCI	Total capital investment
TDC	Total direct cost
TDS	total dissolved solids
TRS	total reducing sugar
UPLC	ultra performance liquid chromatography
WBSG	Weiss brewer’s spent grains
WBSG-25%	15% dry matter Weiss brewer’s spent grains
WBSG-25%	25% dry matter Weiss brewer’s spent grains
WBSG-32%	32% dry matter Weiss brewer’s spent grains
wt.	weight
X2	xylobiose
X3	xylotriose
XOS	xylo-oligosaccharide

List of publications

Process for producing a brewers' spent grain extract and uses thereof.

The invention relates to a process for producing an extract from brewers' spent grain and also to uses of the extract, especially in producing a beer which has been fortified with the extract.

Declaration of contributions

Lukas J Swart wrote the patent manuscript

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1. Introduction

1.1 Reduction of waste and the footprint of beer production

Beer is the most widely consumed alcoholic beverage with global average consumptions reported at 45.7 litre per adult per year in 2016 (The Economist, 2017), making it the third most popular drink after water and tea (Nelson, 2005; Oliver, 2011; Poelmans and Swinnen, 2011). In beer production, brewers' spent grains (BSG) is the residue left after saccharification of the starch from the source material, like malted barley, wheat or rice, and this make up to 85% of brewery's solid waste (IFC, 2007; Levic, Djuragic and Sredanovic, 2010; Mussatto, 2014). The BSG is mostly disposed of without it being dried further, and has a high moisture content, of between 70 and 85 wt.% (Johnson, Paliwal and Cenkowski, 2010; Thiago, Pedro and Eliana, 2014; Lynch, Steffen and Arendt, 2016).

A typical medium-sized brewery produces between 100-200 tonne wet BSG per day (Ishiwaki *et al.*, 2000) and the largest brewery in South Africa is estimated to produce up to 460 tonnes of wet BSG per day ('The South African Breweries', no date; Ramukhwatho, Seetal and Pienaar, 2016). Due to its high moisture content and the bioactive organic matter that it contains (Robertson, l'Anson, *et al.*, 2010), BSG is highly susceptible to spoiling and cannot be stored for long periods (Wang *et al.*, 2014), and, therefore, it is a potentially bio-hazardous waste and its timely disposal can be a logistical challenge to large breweries (Huige, 1994; Thomas, K. R.; Rahman, 2006; Ramukhwatho, Seetal and Pienaar, 2016). Currently, most BSG is either sent to landfill or given away to nearby farmers for animal feed supplement (Schwencke, 2006; Esslinger, 2009; Ben-Hamed, Seddighi and Thomas, 2011; Kerby and Vriesekoop, 2017). Due to the worldwide drive for process sustainability and increasingly more stringent regulations on animal feeds in the United States and European Union, breweries are compelled to investigate alternative solutions for BSG valorisation and disposal (Thomas, K. R.; Rahman, 2006; Esslinger, 2009; LeBlanc, 2014; Kerby and Vriesekoop, 2017).

1.2 Future higher value applications for BSG

Various investigations have shown that BSG as an agro-industrial lignocellulosic biomass can be valorised into wide range of chemicals and energy products such as biofuels (Johnson, Paliwal and Cenkowski, 2010; Pejin *et al.*, 2013; Mussatto, 2014; Nigam, 2017; Skendi, Harasym and Galanakis, 2018). However, the high moisture content and cost of BSG transportation prohibits cost-effective fuel production and thus, higher value applications are required for valorisation of the BSG (Buffington, 2014). BSG is a food grade by-product that can be processed into higher-value health-promoting food products such as nutraceuticals, phytochemicals, sugar replacers, proteins and peptides, which can be extracted and re-

applied in food and beverage formulations (Steiner, Procopio and Becker, 2015; Lynch, Steffen and Arendt, 2016; Monin, 2016; Connolly *et al.*, 2017; Ikram *et al.*, 2017; Abu-ghannam and Balboa, 2018). Its relatively large hemicellulose fraction (>25%), mainly consisting of xylan and arabinan, makes BSG suitable for use as a raw material to produce xylose for xylitol production (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005) and prebiotic oligosaccharides, such as xylo-oligosaccharides (XOS) and arabino-xylo oligosaccharides (AXOS) (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015).

1.3 Hydrothermal treatment process for BSG xylan fractionation

A typical process for obtaining the XOS and xylose from BSG hemicellulose starts with hydrothermal treatment (HTT), during which the BSG is subjected to steam or water in a reactor at high temperatures (150-250 ºC) (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005; Gomez *et al.*, 2015). During HTT, the induced hydrolytic action of water molecules at elevated temperatures causes solubilisation of the hemicellulose fraction into XOS/AXOS oligosaccharides and/or xylose monosaccharides (Mosier, Ladisch and Ladisch, 2002; Bayer, 2007; Ibbett *et al.*, 2011; Kruse and Dahmen, 2015). Depending on the HTT conditions and catalyst additions, hemicelluloses can be selectively solubilised to produce a desired range of XOS oligomers and xylose monomers (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015).

The hemicelluloses hydrolysate is then separated from the solid fraction. For prebiotic XOS production, the hydrolysate is concentrated with membrane separation, followed by selective shortening of the oligomeric chains using enzymes, and then purified and dried (Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). For xylitol production after HTT, the xylose sugar is concentrated and fermented with yeast to xylitol, which is purified through chromatographic separation and crystallisation (Heikkila *et al.*, 1992; Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005). Thus, the HTT step dictates the efficacy of the valorisation process, since the yields and concentrations of the final products are directly dependent on the efficiency of hemicellulose solubilisation in HTT, while the energy consumption of the valorisation process is dependent primarily on processing temperature and on the extent to which the feed BSG had been diluted with water (i.e., solids concentration or dry matter content).

1.4 Hydrothermal treatment process intensification by higher drymatter and lower temperatures

The extent of hemicellulose solubilisation and its selectivity for particular products of interest, are dependent on the process conditions of HTT, including the residence time, temperature (Overend, Chornet and Gascoigne, 1987), solids concentration (Brownell Saddler, 1986;

Cullis, Saddler and Mansfield, 2004; Ibbett *et al.*, 2011; Sui and Chen, 2015) and catalyst used (Chum *et al.*, 1990; Mosier, Ladisch and Ladisch, 2002). The reported parameter ranges for achieving an XOS yield of 61% to 77% from BSG containing 11% dry matter, are temperatures between 185 and 200 °C and process time between 10 and 20 min, using no added catalyst (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). While typical parameter ranges to obtain xylose yields of 85% to 95% from 11% dry matter BSG, are temperatures between 120 to 130 °C and time between 15 and 17 min, using 100-240 mg H_2SO_4 acid/g BSG (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005). Although it is evident that increasing the temperature of the HTT can obtain a yield of XOS in excess of 60% when no additional catalyst is added to BSG, higher temperatures also increase the rate of formation of degradation by-products (inhibitors), in comparison to the rate of xylan solubilisation (Carvalheiro *et al.*, 2005a; Kupiainen, Ahola and Tanskanen, 2014). Lower HTT processing temperatures are therefore advantageous, as these will limit the production of degradation by-products and/or fermentation inhibitors, and reduce the energy requirements in HTT processing.

Higher solids loadings for fractionating BSG in HTT is attractive, as it increases product concentrations, while decreasing energy requirements and process equipment size (Leibbrandt, Knoetze and Görgens, 2011; Galbe and Zacchi, 2012; Modenbach and Nokes, 2012). The energy intensity of low solids loading HTT could cause the global warming impacts to increase substantially (González-García, Morales and Gullón, 2018), unless heat integration is employed to reduce energy consumption (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016). However, higher solids loading in HTT also increases the risk of increasing the production of degradation products and fermentation inhibitors (Modenbach and Nokes, 2012; Jönsson, Alriksson and Nilvebrant, 2013), which reduces the yield of the fractionation process. Higher solids loading also complicates process control and causes poor mixing in conventional stirred vessels (Yang and Tucker, 2013). Typical pressurised, stirred batch reactors, such as the bench scale Parr reactor used in the present study, therefore use a solids loading of less than 15% to minimise limitations in mixing, heat and mass transfer (Yang and Tucker, 2013). Thus purposely designed, high solids equipment that can deal with mixing difficulties is required (Chen, Wu and Lee, 1998; Lee *et al.*, 1998; Shao and Lynd, 2013) for HTT. A steam explosion reactor is more suitable for high (>25%) solids concentration HTT, since direct steam injection assures quick heating of biomass, while rapid depressurisation increases the rate of cooling of products (Schell *et al.*, 1991; Bayer, 2007; Verardi *et al.*, 2012). It has been proposed that, if the negative effects of decreased moisture in HTT processing could be minimised with appropriate equipment, then higher solids loadings can result in similar, or even higher, product yields than those achieved with the lower

solids loadings (Cullis, Saddler and Mansfield, 2004; Mussatto and Roberto, 2006; Roos *et al.*, 2009; Plaza *et al.*, 2017).

1.5 Extremely low acid catalyst addition to aid hydrothermal treament

Supplementation of the hydrothermal reaction with appropriate catalysts could improve overall product yields when operating at lower temperatures (Jensen *et al.*, 2010; Kapoor *et al.*, 2017), thus reducing heat requirements and equipment cost (Fortman, 2009; Tao *et al.*, 2011; Baral and Shah, 2017). On the other hand, adding a catalyst can increase operational costs, since the catalyst is an input cost, and can result in extra waste to consider after HTT (Humbird *et al.*, 2011). Therefore, catalyst dosing must be minimised. To this aim, extremely low acid (ELA) dosing (<0.7wt. % or <20 mg acid/ g) is reported to provide a possible alternative for effective lignocelluloses processing (Liu and Wyman, 2004; Jensen *et al.*, 2010; Shen and Wyman, 2011; Gurgel *et al.*, 2012; Kim, Ryu and Oh, 2016).

1.6 Technoeconomic evaluation: Scale and hydrothermal technology

Two techno-economic studies have been reported for BSG valorisation using HTT, with the addition of 100 mg H_2SO_4 acid/g BSG catalyst; these found xylitol to be the most profitable of the products assessed (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016). However, both studies found the production process to be heat intensive, since heat integration reduced the cost of production by 40%, which can be attributed largely to low dry matter concentration (11.1% dry matter) used in these processes. In another assessment of BSG valorisation by combined production of XOS and ethanol, an equally low dry matter concentration (~11%) was used in a liquid hot water (LHW) HTT process at 210 °C. Results from the environmental impact assessment reveal that the autocatalytic LHW treatment process was a hotspot, with its share in the global process impact ranging between 35% and 55% of all the impacts considered (González-García, Morales and Gullón, 2018). The high energy costs in the three studies can be improved substantially by reducing the water content of BSG during processing, and thus increasing the solids concentration (Leibbrandt, Knoetze and Görgens, 2011); this should be done without negatively affecting product yields.

The scale of the production in the plants for which previous techno-economic assessments for BSG valorisation have been completed (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016), were not realistic, based on the reported estimates of BSG feedstock availability (Ramukhwatho, Seetal and Pienaar, 2016); this results in an underestimation of possible production costs relating to the selected products (Fortman, 2009; Humbird *et al.*, 2011). Due to BSG's high moisture content, the cost of transporting BSG for large-scale, centralised processing, such as fuel production, is prohibitive, and thus, on-site-valorisation to high-value products is required (Buffington, 2014). Therefore, BSG valorisation concepts need

to be co-located at breweries, and utilise the actual scale of BSG availability at brewery sites for techno-economic evaluations.

Both, techno-economic and environmental impact assessments underline the importance of developing improved methods of BSG HTT processing, in particular by increasing the dry matter content and lowering operating temperatures. Therefore, the overall aim of the project was to assess the technical and economic outcomes of producing XOS and xylitol from BSG when such intensification of the BSG HTT process was investigated. This was achieved with the characterisation and optimisation of the HTT process at higher solids loadings and lower temperatures, using two HTT reactor set-ups, (i) a multivariate optimisation in bench scale high pressure stirred reactor using additional ELA dosing, and (ii) scale-up of the preferred HTT process conditions to a pilot scale steam explosion reactor. Processes were optimised in a manner that maximised the yields of the intended products, in particular XOS and xylose (for xylitol production).

1.7 Screw press dewatering of BSG: Efficient hydrothermal process intensification

A pilot scale screw press was used to effectively reduce the moisture content of BSG prior to HTT treatments, as an alternative to drying of BSG with heat or steam, which is economically challenging for industry (Huige, 1994). Mechanical dewatering, such as a screw press, offers a simpler alternative (Scheller and Michel, 2012; Buettner *et al.*, 2015; Weger *et al.*, 2017). Additionally, mechanical dewatering can enrich BSG for lignocelluloses and hemicelluloses (Blinkov et al., 1999; Ishiwaki et al., 2000) by selective removal of soluble salts, proteins and starches, which can furthermore improve the XOS product purity and quality (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977). Moreover screw press treatment of biomass has the advantage of mechanically pretreating the material, resulting in defibrillation and shortening of the fibres, which may enhance the subsequent HTT step (Yan *et al.*, 2014).

1.8 Methodology of this study

Optimisation of the HTT process in a bench-scale, batch, and stirred reactor (Parr) system was undertaken firstly with raw and screw press-dewatered BSG. The effects of temperature, reaction time and ELA catalyst dosing were investigated at a high solids concentrations ranging from 15% to 25% dry matter; process conditions were selected as those likely to maximise the yields of xylose and XOS, for comparison to previously reported optimised LHW HTT at 9-11% dry matter (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005; López-Linares *et al.*, 2019).

Furthermore, the preferred operating regime as identified in bench-testing was scaled-up subsequently to the pilot-scale steam explosion reactor, where the effects of temperature, reaction time and catalysts dosage on the production of XOS from two types of BSG were investigated (WGSB and PBSG), at solids concentrations of 15, 25 and 32% (Lischeske et al., 2016). The experimentally measured output variables included XOS yields, xylobiose and xylotriose fraction in the XOS, monomeric xylose sugar yields, total dissolved solids (TDS), xylan recoveries and the formation of degradation products, i.e. the fermentation inhibitors, furfural, 5-hydroxymethylfurfural (HMF), acetic and formic acids.

Finally process simulations were developed in ASPEN Plus® for three preferred scenarios for BSG valorisation, based on experimental results, for the production of the sweetener xylitol, the prebiotic xylo-oligosaccharide (XOS) or both products simultaneously. Economic models based on the aforementioned process simulations assumed a small scale biorefinery annexed to a brewery, to consider in the financial viability assessments the appropriate production scales and BSG logistics of these scenarios.

1.9 Layout of thesis

The experimental work is discussed first, in Chapter 4 and 5. Chapter 4 describes the batch ELA HTT in a Parr reactor of one feedstock WBSG. In Chapter 5 the screw press dewatering is applied to two types of BSG, WBSG and PBSG. The last section, Chapter 6, combines experimental results obtained in Chapters 4 and 5 into process models for techno-economic assessments of valorisation concepts for producing XOS and xylitol from BSG (Figure 1-1). The underlying conclusions and recommendations are outlined in Chapter 7

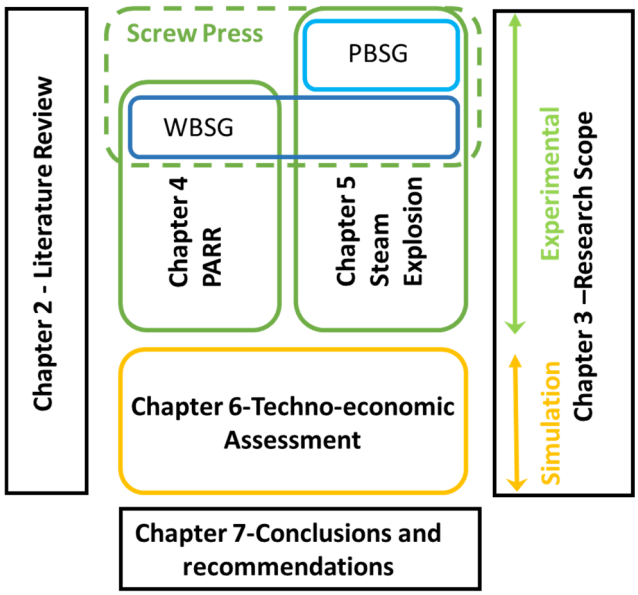


Figure 1-1 Thesis layout

1.10References

Abu-Ghannam, N., Balboa, E., 2018. 9 – Biotechnological, food, and health care applications, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier. https://doi.org/10.1016/B978-0-08-102162-0.00009-5

Baral, N.R., Shah, A., 2017. Comparative techno-economic analysis of steam explosion, dilute sulfuric acid, ammonia fiber explosion and biological pretreatments of corn stover. Bioresour. Technol. 232, 331–343. https://doi.org/10.1016/j.biortech.2017.02.068

Ben-Hamed, U., Seddighi, H., Thomas, K., 2011. Economic returns of using Brewery's spent grain in animal feed. World Acad. Sci. Eng. Technol. 50, 695–698.

Blinkov, S.D., Nemoytin, J.B.B.M., Fedorov, A.L., 1999. Method for processing vegetal raw materials in order to produce pentose hydrolysates essentially containing xylose. WO1999023260.

Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam explosion pretreatment of wood: Effect of Chip Size, Acid, Moisture Content and Pressure Drop. Biotechnol. Bioeng. 28, 792–801. https://doi.org/10.1002/bit.260280604

Buettner, A., Hammer, T., Tackerberg, M., M van Laak, 2015. Method for utilizing spent grain in a brewery and corresponding device US 2015/0068058 A1. US 20150068058.

Buffington, J., 2014. The Economic Potential of Brewer's Spent Grain (BSG) as a Biomass Feedstock. Adv. Chem. Eng. Sci. 308–318.

Carvalheiro, F., Duarte, L.C., Medeiros, R., Gírio, F.M., 2004a. Optimization of Brewery's Spent Grain Dilute-Acid Hydrolysis for the Production of Pentose-Rich Culture Media. Appl. Biochem. Biotechnol. 113–116, 1059–1072. https://doi.org/10.1385/ABAB:115:1-3:1059

Carvalheiro, F., Esteves, M.P., Parajo, J.C., Pereira, H., Gıırio, F.M., 2004b. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91, 93–100. https://doi.org/10.1016/S0960-8524(03)00148-2

Carvalheiro, F., Garrote, G., Parajo, J.C., Pereira, H., Gı, F.M., 2005. Kinetic Modeling of Brewery' s Spent Grain Autohydrolysis. Biotechnol.Prog. 21, 233–243.

Chen, R., Wu, Z., Lee, Y.Y., 1998. Shrinking-Bed Model for Percolation Process Applied to Dilute-Acid Pretreatment/Hydrolysis of Cellulosic Biomass. Appl. Biochem. Biotechnol. 70–72, 37–49.

Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst effects and the combined severity parameter. Appl. Biochem. Biotechnol. 24–25, 1–14. https://doi.org/10.1007/BF02920229

Connolly, A., O'Keeffe, M.B., Nongonierma, A.B., Piggott, C.O., FitzGerald, R.J., 2017. Isolation of peptides from a novel brewers spent grain protein isolate with potential to modulate glycaemic response. Int. J. Food Sci. Technol. 52, 146–153. https://doi.org/10.1111/ijfs.13260

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of Initial Moisture Content and Chip Size on the Bioconversion Efficiency of Softwood Lignocellulosics. Biotechnol. Bioeng. 85, 413–421. https://doi.org/10.1002/bit.10905

Dávila, J.A., Rosenberg, M., Cardona, C.A., 2016. A biorefinery approach for the production of xylitol, ethanol and polyhydroxybutyrate from brewer's spent grain. AIMS Agric. Food 1, 52–66. https://doi.org/10.3934/agrfood.2016.1.52

Esslinger, H.M., 2009. Handbook of Brewing, Wiley-VCH Verlag GmbH. https://doi.org/10.1017/CBO9781107415324.004

Finley, J.W., Walker, C.E., Hautala, E., 1976. Utilization of Press Water from Brewers Spent Grains. J. Sci. Food Agric. 27, 655–660.

Fortman, J.A., 2009. Techno-economic comparison of hot water and dilute acid pretreatment for biochemical production of ethanol from corn stover and evaluation of alternative scenarios to purchasing cellulase enzymes. Iowa State University.

Galbe, M., Zacchi, G., 2012. Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy 46, 70–78. https://doi.org/10.1016/j.biombioe.2012.03.026

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

González-García, S., Morales, P.C., Gullón, B., 2018. Estimating the environmental impacts of a brewery waste–based biorefinery: Bio-ethanol and xylooligosaccharides joint production case study. Ind. Crops Prod. 123, 331–340. https://doi.org/10.1016/j.indcrop.2018.07.003

Gullón, P., González-Muñoz, M.J., Parajó, J.C., 2011. Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. Bioresour. Technol. 102, 6112–6119. https://doi.org/10.1016/j.biortech.2011.02.059

Gurgel, L.V.A., Marabezi, K., Zanbom, M.D., Curvelo, A.A.D.S., 2012. Dilute acid hydrolysis of sugar cane bagasse at high temperatures: A Kinetic study of cellulose saccharification and glucose decomposition. Part I: Sulfuric acid as the catalyst. Ind. Eng. Chem. Res. 51, 1173–1185. https://doi.org/10.1021/ie2025739

Heikkila, H., Nurmi, J., Rahkila, L., Toyryla, M., 1992. Method for the production of xylitol. 5081026.

Huige, N.J., 1994. Handbook of Brewing: In Food Science and Technology, in: Hardwick, W.A. (Ed.), Marcel Dekker Inc. CRC Press, pp. 501–550). https://doi.org/10.1002/9780470290118

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., Dudgeon, D., 2011. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol, National Renewable Energy Laboratory.

Ibbett, R., Gaddipati, S., Davies, S., Hill, S., Tucker, G., 2011. The mechanisms of hydrothermal deconstruction of lignocellulose : New insights from thermal – analytical and complementary studies. Bioresour. Technol. 102, 9272–9278. https://doi.org/10.1016/j.biortech.2011.06.044

IFC, 2007. Environmental, Health, and Safety Guidelines for Breweries.

Ikram, S., Huang, L., Zhang, H., Wang, J., Yin, M., 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 00, 1–11. https://doi.org/10.1111/1750-3841.13794

Ishiwaki, B.N., Murayama, H., Awayama, H., Kanauchi, O., Sato, T., 2000. Development of High Value Uses of Spent Grain by Fractionation Technology Q uarterly. MBAA Tech. Q. 37, 261–265.

Jensen, J.R., Morinelly, J.E., Gossen, K.R., Brodeur-Campbell, M.J., Shonnard, D.R., 2010. Effects of dilute acid pretreatment conditions on enzymatic hydrolysis monomer and oligomer sugar yields for aspen, balsam, and switchgrass. Bioresour. Technol. 101, 2317–2325. https://doi.org/10.1016/j.biortech.2009.11.038

Johnson, P., Paliwal, J., Cenkowski, S., 2010. Issues with utilisation of brewers' spent grain. Stewart Postharvest Rev. 6, 1–8. https://doi.org/10.2212/spr.2010.4.2

Jönsson, L.J., Alriksson, B., Nilvebrant, N.-O., 2013. Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnol. Biofuels 6, 16. https://doi.org/10.1186/1754-6834-6-16

Kapoor, M., Soam, S., Agrawal, R., Gupta, R.P., Tuli, D.K., Kumar, R., 2017. Pilot scale dilute acid pretreatment of rice straw and fermentable sugar recovery at high solid loadings. Bioresour. Technol. 224, 688–693. https://doi.org/10.1016/j.biortech.2016.11.032

Kerby, C., Vriesekoop, F., 2017. An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3, 1–12. https://doi.org/10.3390/beverages3020024

Kim, T.H., Ryu, H.J., Oh, K.K., 2016. Low acid hydrothermal fractionation of Giant Miscanthus for production of xylose-rich hydrolysate and furfural. Bioresour. Technol. 218, 367–372. https://doi.org/10.1016/j.biortech.2016.06.106

Kruse, A., Dahmen, N., 2015. Water - A magic solvent for biomass conversion. J. Supercrit. Fluids 96, 36–45. https://doi.org/10.1016/j.supflu.2014.09.038

Kupiainen, L., Ahola, J., Tanskanen, J., 2014. Kinetics of Formic Acid-catalyzed Cellulose Hydrolysis. BioResources 9, 2645–2658. https://doi.org/10.15376/biores.9.2.2645-2658

LeBlanc, T., 2014. Why the FDA's Spent Grain Policy Nearly Raised the Cost of Your Beer [WWW Document]. Mod. Farmer. URL https://modernfarmer.com/2014/07/great-spent-grain-debacle/ (accessed 9.10.18).

Lee, Y.Y., Iyer, P., Xiang, Q., Hayes, J., 1998. Kinetic and Modeling Investigation to Provide Design Guidelines for the NREL Dilute- Acid Process Aimed at Total Hydrolysis / Fractionation of Lignocellulosic Biomass July 1998 Kinetic and Modeling Investigation to Provide Design Guidelines for the NREL D. NREL Tech. Rep.

Leibbrandt, N.H., Knoetze, J.H., Görgens, J.F., 2011. Comparing biological and thermochemical processing of sugarcane bagasse: An energy balance perspective. Biomass and Bioenergy 35, 2117–2126. https://doi.org/10.1016/j.biombioe.2011.02.017

Levic, J., Djuragic, O., Sredanovic, S., 2010. Use of new feed from brewery by-products for breeding layers. Rom. Biotechnol. Lett. 15, 5559–5565.

Liu, C., Wyman, C.E., 2004. The Effect of Flow Rate of Very Dilute Sulfuric Acid on Xylan, Lignin, and Total Mass Removal from Corn Stover. Ind. Eng. Chem. Res. 43, 2781–2788. https://doi.org/10.1021/ie030754x

López-Linares, J.C., García-Cubero, M.T., Lucas, S., González-Benito, G., Coca, M., 2019. Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production. Chem. Eng. J. 368, 1045–1055. https://doi.org/10.1016/j.cej.2019.03.032

Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. J. Inst. Brew. 4, 553–568. https://doi.org/10.1002/jib.363

Mathias, T.R.S., de Mello, P.P.M., Eliana, F.C.S., 2014. Solid wastes in brewing process: A review. J. Brew. Distill. 5, 1–9. https://doi.org/10.5897/JBD2014.0043

Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment-A review. Biotechnol. Bioeng. 109, 1430–1442. https://doi.org/10.1002/bit.24464

Monin, T., 2016. Life Refreshment - Pilot for Environmentally Friendly, Efficient, Sustainable and Healthy products development: LIFE15 ENV/BE/000267.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R., 2002. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. 79, 610–618. https://doi.org/10.1002/bit.10316

Mussatto, S.I., 2014. Brewer's spent grain: A valuable feedstock for industrial applications. J. Sci. Food Agric. 94, 1264–1275. https://doi.org/10.1002/jsfa.6486

Mussatto, S.I., Moncada, J., Roberto, I.C., Cardona, C.A., 2013. Techno-economic analysis for brewer's spent grains use on a biorefinery concept: The Brazilian case. Bioresour. Technol. 148, 302–310. https://doi.org/10.1016/j.biortech.2013.08.046

Mussatto, S.I., Roberto, I.C., 2006. Chemical characterization and liberation of pentose sugars from brewer's spent grain. J. Chem. Technol. Biotechnol. 81, 268–274. https://doi.org/10.1002/jctb.1374

Mussatto, S.I., Roberto, I.C., 2005. Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J. Sci. Food Agric. 85, 2453–2460. https://doi.org/10.1002/jsfa.2276

Nelson, M., 2005. The Barbarian's Beverage: A History of Beer in Ancient Europe. Routledge.

Nigam, P.S., 2017. An overview: Recycling of solid barley waste generated as a by-product in distillery and brewery. Waste Manag. 62, 255–261. https://doi.org/10.1016/j.wasman.2017.02.018

Oliver, G., 2011. The Oxford Companion to Beer. Oxford University Press.

Olsson, L., 2007. Biofuels, Advances in Biochemical engineering/Biotechnology. https://doi.org/10.1016/j.jbiotec.2007.09.006

Overend, R., Chornet, E., Gascoigne, J.A., 1987. Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments [and Discussion], Philosophical Transactions of The Royal Society A: Mathematical, Physical and Engineering Sciences. https://doi.org/10.1098/rsta.1987.0029

Pejin, J.D., Radosavljević, M.S., Grujić, O.S., Mojović, L.V., Kocić-Tanackov, S.D., Nikolić, S.B., Djukić-Vuković, A.P., 2013. Possible application of brewer's spent grain in biotechnology | Mogućnosti primene pivskog tropa u biotehnologiji. Hem. Ind. 67, 277–291. https://doi.org/10.2298/HEMIND120410065P

Plaza, P.E., Gallego-Morales, L.J., Peñuela-Vásquez, M., Lucas, S., García-Cubero, M.T., Coca, M., 2017. Biobutanol production from brewer's spent grain hydrolysates by Clostridium beijerinckii. Bioresour. Technol. 244, 166–174. https://doi.org/10.1016/j.biortech.2017.07.139

Poelmans, E., Swinnen, J.F.M., 2011. From Monasteries to Multinationals (and Back): A Historical Review of the Beer Economy, LICOS Discussion Paper, No. 294, LICOS, Leuven.

Ramukhwatho, F., Seetal, A., Pienaar, H., 2016. NATSURV 1: Water and Wastewater Management in the Malt Brewing Industry (Ed 2) WRC Report No. TT 676/16. Pretoria.

Robertson, J.A., I'Anson, K.J.A., Treimo, J., Faulds, C.B., Brocklehurst, T.F., Eijsink, V.G.H., Waldron, K.W., 2010. Profiling brewers' spent grain for composition and microbial ecology at the site of production. LWT - Food Sci. Technol. 43, 890–896. https://doi.org/10.1016/j.lwt.2010.01.019

Roos, A.A., Persson, T., Krawczyk, H., Zacchi, G., Stålbrand, H., 2009. Extraction of water-soluble hemicelluloses from barley husks. Bioresour. Technol. 100, 763–769. https://doi.org/10.1016/j.biortech.2008.07.022

SAB, 2012. The South African Breweries.

Schell, D.J., Torget, R., Walter, A.P.P.J., Grohmann, K., Hinman, N.D., 1991. A Technical and Economic Analysis of Acid-Catalyzed Steam Explosion and Dilute Sulfuric Acid Pretreatments Using Wheat Straw or Aspen Wood Chips 28.

Scheller, L., Michel, R., 2012. Brewing Beer – efficient and sustainable technologies in regard to energy and raw material input, in: ERSCP Workshop, Bregenz, 3 May 2012. GEA Brewery Systems Huppmann Tuchenhagen, Bregenz, pp. 1–38.

Schwencke, K. V, 2006. Sustainable, Cost-Effective, and Feasible Solutions for the Treatment of Brewers' Spent Grains. MBAA Tech. Q. 43, 199–202.

Shao, X., Lynd, L., 2013. Kinetic modeling of xylan hydrolysis in co- and countercurrent liquid hot water flow-through pretreatments. Bioresour. Technol. 130, 117–124. https://doi.org/10.1016/j.biortech.2012.11.109

Shen, J., Wyman, C.E., 2011. A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. Bioresour. Technol. 102, 9111–9120. https://doi.org/10.1016/j.biortech.2011.04.001

Skendi, A., Harasym, J., Galanakis, C.M., 2018. 7 – Recovery of high added-value compounds from brewing and distillate processing by-products, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier Science. https://doi.org/10.1016/B978-0-08-102162-0.00007-1

Steiner, J., Procopio, S., Becker, T., 2015. Brewer's spent grain: source of value-added polysaccharides for the food industry in reference to the health claims. Eur. Food Res. Technol. 303–315. https://doi.org/10.1007/s00217-015-2461-7

Stiles, R.S., Herbert, H.S., 1977. Centrifugal Separation, Isolation and Characteristics of Brewery Spent Grains and Press Liquor. MBAA Tech. Q. 14, 21–34.

Sui, W., Chen, H., 2015. Study on loading coefficient in steam explosion process of corn stalk. Bioresour. Technol. 179, 534–542. https://doi.org/10.1016/j.biortech.2014.12.045

Taleb, N.N., 2010. The Bed Of Procrustes: Philosophical and Practical Aphorisms, 2nd Editio. ed. Random House.

Tao, L., Aden, A., Elander, R.T., Pallapolu, V.R., Lee, Y.Y., Garlock, R.J., Balan, V., Dale, B.E., Kim, Y., Mosier, N.S., Ladisch, M.R., Falls, M., Holtzapple, M.T., Sierra, R., Shi, J., Ebrik, M.A., Redmond, T., Yang, B., Wyman, C.E., Hames, B., Thomas, S., Warner, R.E., 2011. Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass. Bioresour. Technol. 102, 11105–11114. https://doi.org/10.1016/j.biortech.2011.07.051

The Economist, 2017. Around the world, beer consumption is falling - Daily chart [WWW Document]. Econ. URL https://www.economist.com/graphic-detail/2017/06/13/around-the-world-beer-consumption-is-falling (accessed 9.10.18).

Thomas, K.R., Rahman, P.K.S.M., 2006. Brewery wastes. Strategies for sustainability. A review. Asp. Appl. Biol. 80, 147–153.

Verardi, A., Bari, I. De, Ricca, E., Calabrò, V., 2012. Hydrolysis of Lignocellulosic Biomass: Current Status of Processes and Technologies and Future Perspectives, in: Lima, M.A.P. (Ed.), Bioethanol. InTech, pp. 96–122. https://doi.org/10.5772/23987

Wang, B., Luo, Y., Myung, K.H., Liu, J.X., 2014. Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grains. Asian-Australasian J. Anim. Sci. 27, 832–840. https://doi.org/10.5713/ajas.2013.13668

Weger, A., Jung, R., Stenzel, F., Hornung, A., 2017. Optimized Energetic Usage of Brewers' Spent Grains. Chem. Eng. Technol. 40, 306–312. https://doi.org/10.1002/ceat.201600186

Yan, Q., Miazek, K., Grande, P.M., Domínguez de María, P., Leitner, W., Modigell, M., 2014. Mechanical pretreatment in a screw press affecting chemical pulping of lignocellulosic biomass. Energy & Fuels 28, 6981–6987. https://doi.org/10.1021/ef501706w

Yang, B., Tucker, M., 2013. Laboratory Pretreatment Systems to Understand Biomass Deconstruction, in: E., C., Wyman (Eds.), Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals. John Wiley & Sons Inc., pp. 489–521. https://doi.org/10.1002/9780470975831.ch23

2. Literature Review

The review focuses on hydrothermal processes for extracting fractions of XOS and/or xylose from BSG hemicellulose. Technologies involved in XOS and xylitol production that need to be considered in techno-economic assessments for valorisation concepts of BSG will also be considered.

2.1 Beer brewing and BSG production

Beer is the most widely consumed alcoholic beverage and is considered to be the third most popular drink after water and tea (Oliver, 2011; Poelmans and Swinnen, 2011). Brewing beer dates back to earliest recordings of civilisations with recipes dating as far back to 5000 BC in ancient Egypt, Mesopotamia and China, while the oldest uninterrupted beer producing facility, since at least 1040 AD, is Brauerei Weihenstephan in Germany (Nelson, 2005).

2.1.1 The beer brewing process

BSG is the residue produced by beer production after mashing or saccharification of the starch from grains, such as malted barley, wheat, sorghum or rice (Bamforth, 2000; Mussatto, Dragone and Roberto, 2006). Malted sorghum and millet are commonly used for traditional African brews, since they are suited for cultivation in African climates (Palmer, 1992). A general depiction of the beer production process is shown in Figure 2-1.

Before the mashing step, the dried malted grains are milled in a hammer or roller mill, to expose the starchy endosperm. Mashing is an enzymatic hydrolysis process that is conducted for 5-60 minutes, at temperatures between 35 and 78°C, and produces mainly sugars from starch for beer fermentation, while also solubilising proteins, β-glucan and minerals (Briggs *et al.*, 2004; Mussatto, Dragone and Roberto, 2006; Esslinger, 2009). About 95 wt.% of initial starch can be hydrolysed, while 60-70 wt.% of the ash and 35-55 wt.% of the barley proteins can be removed simultaneously. It is reported that less than 0.7% of the original (5-8 wt.%) arabinoxylan fraction in barley malt is water soluble, and most of the arabinoxylan fraction is therefore left in the insoluble BSG (Briggs *et al.*, 2004; Celus, 2008).

After mashing has been completed, the BSG is removed as filter cake by lautering or mash filtration, leaving a clear sugar wort. All of the insoluble mashing products and grain residues are retained in the filter cake, including empty aleurone cell walls, the barley seed husk, pericarp and coat, and disposed of as BSG. BSG can represent between 20 and 30% (dry basis) of the original cereal or malt used, depending on the type of malt, brew recipe and adjuncts added (Briggs *et al.*, 2004; Mussatto, Dragone and Roberto, 2006; Esslinger, 2009; Skendi, Harasym and Galanakis, 2018). BSG moisture content and chemical composition vary as a result of the grain properties and the hydrolysis/separation methods and equipment used

(Huige, 1994; Fumi *et al.*, 2011). BSG that has been separated from wort in a mash strainer or lauter tun has a moisture content above 80 wt.%, while that separated with filter presses achieves 73-75 wt.% final moisture content, which is more suitable for handling (Schneider *et al.*, no date; Huige, 1994).

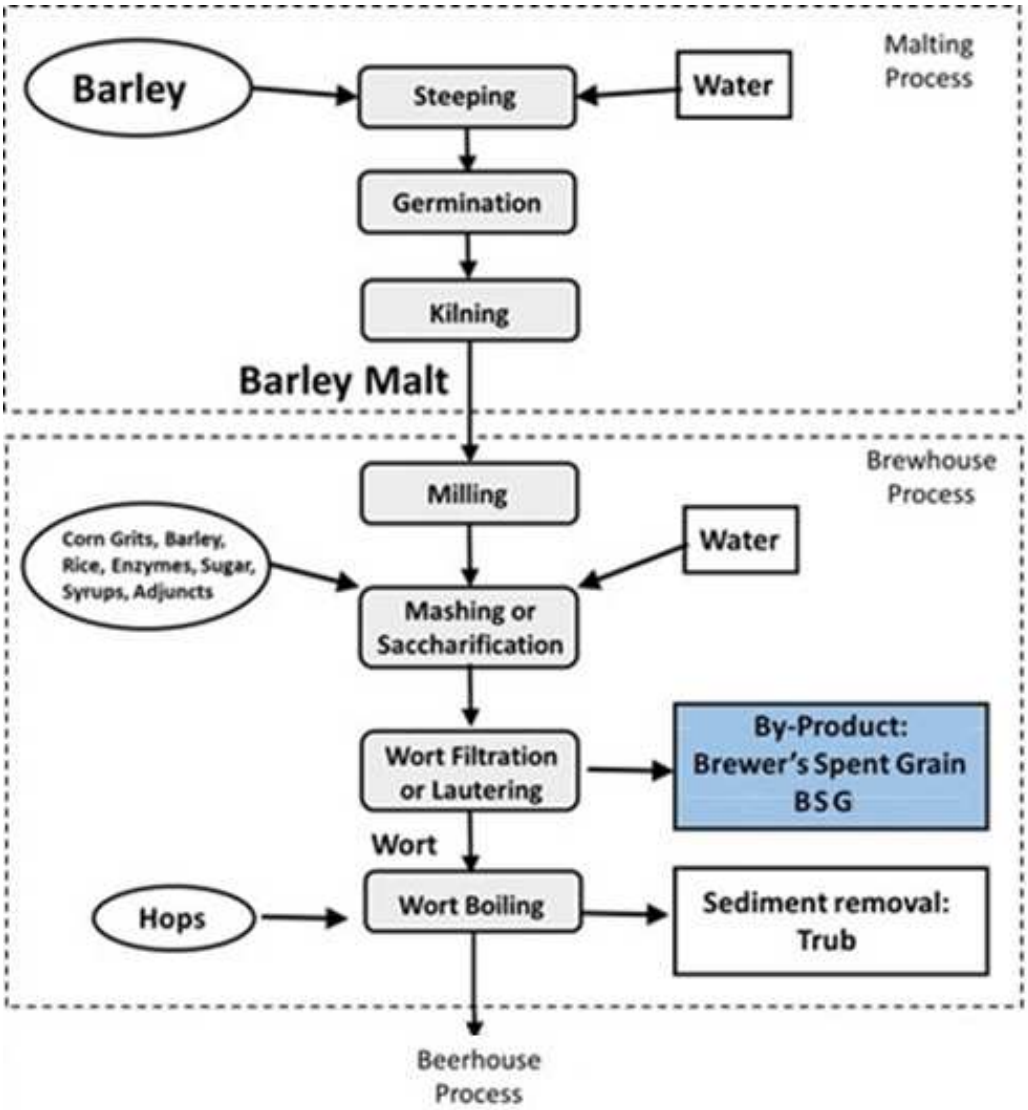


Figure 2-1 Generic process flow diagram in beer production (Adapted (Mussatto, Dragone and Roberto, 2006);(Ramukhwatho, Seetal and Pienaar, 2016))

Other solids that are removed downstream from mashing in the beer process include spent hops (0.08-0.17 kg/100 L beer), trub sediment (0.20-0.40 kg/100 L beer) from the wort boiling and surplus yeast (0.23-0.31 kg/100 L beer) from fermentation (Mathias et al., 2014; Ramukhwatho et al., 2016; Thomas and Rahman, 2006; Huige, 1994). These brewery organic wastes streams, including BSG, have a high biochemical and chemical oxygen demand (BOD/COD) and are classified as high strength organic wastes (Gunes *et al.*, 2019); all these streams can end up mixed together with the BSG for disposal.

2.1.2 BSG availability from beer production

2.1.2.1 Beer production ratio to BSG and estimated availability

In 2017, global average beer consumption stood at 45.7 l per adult per year (The Economist, 2017). Inevitably, around 15-33 kg wet BSG per 100 l beer is produced as a by-product, and makes up 85% of solid waste generated during beer production (Thomas, K. R.; Rahman, 2006; Celus, 2008; Mussatto, 2014; Thiago, Pedro and Eliana, 2014). This means breweries worldwide produce around 10-40 million tons of BSG annually. The largest single-site brewery in the world is the Coors Brewery, located in Golden, Colorado, United States, which produces an estimated 130 000 ton dry BSG as by-product per year (Garrido, 2012). In South Africa, only the two largest breweries produce more than 30 000 tons dry BSG per year. An average-sized brewery produces around 200 tons wet BSG per day, or around 10 000-20 000 tons dry BSG per year (Ishiwaki *et al.*, 2000; Ramukhwatho, Seetal and Pienaar, 2016).

2.1.2.2 Disposal of BSG in breweries

Before the energy crisis in the late 1970s, most large breweries, especially in the United States, dried their BSG to under 14 wt.% moisture content, and stored and sold it as commoditised brewers' dried grains, typically at US$390/ton$_{\text{crude protein}}$ (Stationary, 1981; Huige, 1994). Current solutions for handling BSG vary between breweries and countries, and according to location, production capacity and available markets, such as farmers. Studies of breweries, both multinational commercial and craft brewers, found that most breweries dispose of BSG to landfill, or, if available, to local farmers free of charge, as the simplest and most cost-effective solution (Schwencke, 2006; Acacio *et al.*, 2011; Rosa and Beloborodko, 2015; Kerby and Vriesekoop, 2017). BSG is commonly transported from breweries by truck for disposal, mostly as feed supplement for ruminants at no charge, with only transportation cost determining its cost (Huige, 1994; Esslinger, 2009; Ben-Hamed, Seddighi and Thomas, 2011; Kerby and Vriesekoop, 2017). Yet, the application of BSG as feed supplement is limited by its highly fibrous content to 30% of ruminant feed (Mahnken, 2010; Skendi, Harasym and Galanakis, 2018). Furthermore, the high rate of spoiling limits BSG storage (Robertson, et al. 2010; Wang et al. 2014), while the cost of transporting the wet material (Buffington, 2014) limits its overall cost-effective application as feed to within 10 to 50 km of a brewery (Ben-Hamed et al., 2011; Kerby and Vriesekoop, 2017; Rosa and Beloborodko, 2015). The drive to improve resource efficiency in brewing (Monin, 2016), stricter regulations on animal feeds (LeBlanc, 2014; Kerby and Vriesekoop, 2017; Ravindran *et al.*, 2018), and declining feedlot numbers in developed countries necessitate that breweries find alternative disposal methods for BSG (Ishiwaki *et al.*, 2000; Weger *et al.*, 2014, 2017).

2.2 Composition of BSG

2.2.1 Chemical composition

BSG is considered to be a lignocellulosic material, despite a relatively high protein content (>20%) compared to other lignocelluloses (Mussatto, Dragone and Roberto, 2006; Steiner, Procopio and Becker, 2015). Most lignocellulosic materials comprise mainly lignin, polysaccharides hemicellulose and cellulose, with small amounts of other components, including lipids, oils, waxes and minerals or ash (Isikgor and Becer, 2015; Bajpai, 2016). Most reported BSG compositional analyses are done according to standardised methods, such as (Technical Association of the Pulp and Paper Industry) TAPPI and (American Society for Testing and Materials) ASTM for woody materials, or Laboratory Analytical Procedures by National Renewable Energy Laboratory (NREL) for lignocellulosic biomass (Sluiter et al., 2010).

Although variation in the composition of BSG is expected for different breweries, as a result of different starting materials and brew recipes, composition analyses of replicate samples from a single brewery were found to be consistent (Santos et al., 2003). An important study on the composition of BSG compared 10 large commercial breweries in the European Union and South Africa, and found that the only significant differences were related to protein and starch content (Robertson et al., 2010a). This finding is to be expected, since minimising the residual starch from starch hydrolysis is a critical brewing parameter, which is determined by the effectiveness of the mashing process that converts starch polymers into maltose sugars (Stiles and Herbert, 1977; Esslinger, 2009; Science, 2009). Nevertheless, BSG compositions reported across different studies vary considerably as a result of different compositional analysis methods applied and the diverse speciality malts and recipes used in small craft brews. As shown in Table 2-1, hemicellulose content varied from 16.5 to 50 wt.%, and lignin from 4 to 34.8 wt.%.

Table 2-1 Main components of BSG reported by various studies

Component	Proteins	Starch	Cellulose	Hemi-cellulose	Lignin	Ash	Lipids
Meneses et al., (2013)	24.7	n.d.	21.7	19.3	19.4	4.2	n.d.
Wilkinson et al., (2016)	27.9	1.2	22.1	19.3	10.7	2.7	6.3
Kemppainen et al., (2016)	22.6	n.d.	18.1	25.2	19.6	4.1	11.4
Borel et al., (2018)	22.5	n.d.	15.1	50.2	29.4	3.2	5.3
Mata et al., (2015)	n.d.	n.d.	6.09	39.7	34.8	4.4	5.4
Jay et al., (2008)	24	10.6	17	39	4	n.d.	6
Gómez et al., (2015)	26.8	9.4	24.9	21.5	22.8	3.0	n.d.
Procentese et al., (2018)	n.d.	n.d.	16,8	18.5	21.5	n.d.	n.d.
Amorim et al., (2019)	19.2	n.d.	17.5	16.5	27.0	4.5	n.d.
Maiti et al., (2018)	n.d.	12.5	17.1	32.5	13.4	7.8	3.5

n.d; Not determined

The highest starch content reported in Table 2-1 is 12.5 wt.%, which still represents more than 90% hydrolysis of the starch originally present in barley malt. In nature, hemicellulose is the second-most-abundant polysaccharide after cellulose and, in some cases, it can make up more than 30% of BSG (Table 2-1). The primary polysaccharide present in BSG hemicellulose is xylan, which is a polymer consisting of a linear xylose backbone substituted with arabinan, acetyl and phenolic groups (Kabel *et al.*, 2002)(Table 2-2).

Table 2-2 BSG hemicelluloses composition

Components of BSG hemicellulose	Xylan	Arabinan	Galactan	Mannan	Rhamnan	Fucose	Uronic acids
mass%[a]	16.6	7.0	1.1	0.5	0.1	n.d.	n.d.
mol%[b]	29.84	15.36	2.0	1.24	0.2	0.11	12.6

[a] (Kemppainen *et al.*, 2016); [b] (Jay et al., 2008)

Xylan is more branched and lower in crystallinity than cellulose, which makes xylan polymers more readily hydrolysable (Mussatto and Teixeira, 2010). The xylan polymer consists of anhydrous xylose units that are linked by β-(1 → 4) glycosidic bonds (Kontturi, 2015; Bajpai, 2016). Xylan polymers have a low degree of polymerisation (DP<200 units), compared to cellulose (DP from 800-20 000). Xylan polymers with DP<30 are generally soluble in water, and the addition of more hydrophilic groups can increase the solubility of xylan polymers with higher DP (Hettrich *et al.*, 2017). The arabinosyl bonds in the BSG hemicelluloses structures are very susceptible to hydrolysis, and release arabinose more readily than xylose (Beldman, Hennekam and Voragen, 1987). The feruloylated arabinoxylans found in BSG are mostly the leftover cell walls of barley (Laine *et al.*, 2015).

Lignin composition and content varies across types of biomass, and reported values for BSG range from as low as 4% to 34.8 wt.% (Table 2-1). Lignin in BSG consists of mostly guaiacyl units, and between 25 and 30% syringyl units that are linked with hydroxycinnamic, ferulic and coumaric acids (Niemi *et al.*, 2012; Rencoret *et al.*, 2015).

Table 2-1 indicates that BSG crude protein content varies between 19.2 and 27.9 wt.% of dry BSG, while barley grains contain between 10-17 wt.% crude protein before mashing (Briggs *et al.*, 2004; Baik and Ullrich, 2008). During the mashing process, between 35 and 55% of the barley proteins are hydrolysed and extracted in the sugar wort (Briggs *et al.*, 2004; Baik and Ullrich, 2008). Mashing and beer recipes can affect the resulting BSG protein content, yet different BSG's can show similar amino acid profiles (Robertson, I'Anson, *et al.*, 2010). The profiles of amino acid of BSG proteins that glutamine accounts for about 18.5% of the protein, while 34% is essential amino acids (Celus, 2008), which is similar to values reported for barley husks (Stiles and Herbert, 1977). Table 2-3 shows that when compared to untreated barley

husks, hydrophobic amino acids are concentrated in BSG as a result of hydrolysis during the mashing process, while basic amino acids are selectively removed.

Table 2-3 Amino acid profiles of BSG proteins and other brewery by-products

Amino acid (wt.%)	BSG[a]	BH[b]	BPL[c]
Basic amino acids	9.7	14.6	**11.5**
Hydrophobic amino acids	51.7	45.3	39.0
Hydrophilic amino acids	38.6	37.4	41.1
Essential amino acids*	34.9	39.4	35.8

[a] Brewers spent grains (Celus, 2008)
[b] Barley husks (Stiles and Herbert, 1977)
[c] Brewer's grains press liquid insolubles (Finley, Walker and Hautala, 1976)

Selective changes in the amino acid profiles have also been reported for mechanically separated BSG protein fractions: Amino acid profiles of a protein concentrate (BPL) obtained from BSG with screw press which contained 54% crude protein content (Finley, Walker and Hautala, 1976), showed concentration of hydrophilic amino acids and a significantly reduced proline content. This would indicate the protein is more bound to the remaining insoluble solids in BSG.

2.2.2 Microbiological composition and spoilage of BSG

As a result from the mashing process, the bioactivity of BSG is very high from the original cereal microflora, which quickly leads to BSG spoilage in warm climates, where mold and yeast populations increase exponentially over a few hours (Robertson, et al. 2010; Wang et al. 2014). A study on the micro-organisms in samples of fresh BSG from different commercial breweries found mold and yeast to be lower in count than bacteria, while aerobic thermophiles were the most abundant at around 10^7 cells/g fresh weight in some samples (Robertson, l'Anson, *et al.*, 2010).

BSG is released from the brewing process at more than 40 °C and as a result of its residual sugars, enzymes, nutrients and high moisture it is ideal for further enzymatic degradation and microbial growth (Mussatto, Dragone and Roberto, 2006; Johnson, Paliwal and Cenkowski, 2010; Robertson, lAnson, *et al.*, 2010). The limited heat loss to the environment and limited air in the bulk BSG promotes anaerobic microbial growth (Robertson, l'Anson, *et al.*, 2010). A study by Wang et al. (2014) reported that the neutral detergent fibre (NDF) content of BSG reduced from 59.8% to 50.6% after three days storage at 25 °C, and resulted in an 19.8% overall loss of the dry mass. Moreover, even storage at temperature of 5 °C did not prevent dry matter loss over a day (Wang *et al.*, 2014). These findings are consistent with the report by Robertson et al., (2010b) that found phenolic acids decreased significantly in the first three

days of storage at 4 °C and 20 °C and even autoclave treatment beforehand did not prevent mass loss due to microbial degradation.

2.2.3 Preservation and drying of BSG

Avoiding microbial degradation is key in sustainable utilisation or valorisation of BSG (Huige, 1994; Briggs *et al.*, 2004; Esslinger, 2009). Chemical preservatives like potassium sorbate or acidifying agents like citric, acetic, formic or benzoic acids (Mussatto, Dragone and Roberto, 2006) have been reported for preservation of BSG. Bacterial and enzymatic treatments have also been reported, which improve the growth of beneficial microbes, while reportedly preserving the nutritional value of BSG for animal feed up to 14 days (Marston, 2007). Without adding any preservative, freezing wet BSG was found to be most effective method for preserving crude protein content, when compared to oven- or freeze-drying (Santos *et al.*, 2003); however, a subsequent thawing still might cause losses in arabinose content (Bartolomé *et al.*, 2002).

Typical mechanical dewatering such as filter presses can produce BSG with a moisture content of approx. 74 wt. %, which improves handling (Huige, 1994; El-Shafey *et al.*, 2004; Machado *et al.*, 2016). However, moisture contents of lower than 14 wt.%, are required to prevent microbial growth (Huige, 1994; Tang, 2003). Mechanical dewatering is combined with the thermal drying of BSG to achieve such moisture contents. Industrial dryers include steam tube, flash type, rotary drum or disk dryers, with direct or indirect heating with superheated steam (Ström, no date; Stroem, Desai and Hoadley, 2009; Aboltins and Palabinskis, 2015; Romdhana, Bonazzi and Esteban-Decloux, 2015; Sanni and Fakunle, 2016). Rotary drums to dry BSG reported require between 1.2 to 1.5 tonne steam per 1 tonne wet BSG (Huige, 1994). Thermal drying at harsh conditions can degrade proteins and generate off flavours, causing irreversible aggregation in cellulosic fibrils and decreasing the pore size of fibres (Huige, 1994; Skendi et al., 2018, Fernandes Diniz et al., 2004). To lower the degradation and to avoid off flavour spoilage in BSG a few alternatives options were proposed, such as a novel drying systems that uses moderate heat (Krøll, 2017) or spray drying (‘Method of treating spent grain US 3721568 A’, 1973).

2.3 High value products from BSG

The most prevalent solution for BSG disposal is application as animal feed or supplement. Yet various studies reported on other applications of BSG, such as fuels (Liguori *et al.*, 2015; Plaza *et al.*, 2017) or bio-based chemicals (Skendi et al., 2018). Buffington (2014) found that fuel production from BSG does not recover costs of transportation BSG, due to its high moisture content, and concluded that higher value applications are required. As a food grade waste stream, the highest value application for BSG is with applications in the food industry (Report,

2008; Steiner, Procopio and Becker, 2015; Lynch, Steffen and Arendt, 2016; Monin, 2016; Ikram *et al.*, 2017; Skendi, Harasym and Galanakis, 2018); this research area showed exponential growth from 2005 in published literature (Abu-ghannam and Balboa, 2018).

2.3.1 BSG for food product applications

Historically, in Europe, breweries in small towns supplied local bakeries with fresh BSG, which was reprocessed into bread and other foods. This tradition is said to have been lost after the Second World War, when only a few breweries and bakeries continued baking with BSG (Pauli, 2015; Rosa and Beloborodko, 2015; Kerby and Vriesekoop, 2017). Literature reports that direct addition of BSG in bread formulation, up to a maximum of 10% flour supplementation, has good organoleptic attributes (Waters *et al.*, 2012; Letters and Cluj-napoca, 2014). The resulting darkened colour, changes in texture due to increased fibre, and the bitter taste that some phenolic compounds, fatty acids and peptides introduce, however, limited the use of the whole BSG in baking (Wilhelmson *et al.*, 2009; Cook, 2011). Selective extraction of certain compounds for introduction in food and beverage formulations, through BSG fractionation, can overcome these drawbacks (Lynch, Steffen and Arendt, 2016). Recent reports on BSG application in the food industry focus on novel high-value health-promoting components, with BSG being applied in the food industry as food additives, supplements or nutraceuticals (Report, 2008; Steiner, Procopio and Becker, 2015; Lynch, Steffen and Arendt, 2016; Monin, 2016; Ikram *et al.*, 2017; Skendi, Harasym and Galanakis, 2018). One novel product that can be produced from BSG hemicellulose is a group of carbohydrates and soluble dietary fibres, known as XOS.

2.3.1.1 Xylo-oligosaccharides (XOS) as a prebiotic

BSG hemicellulose consists mainly of arabinose substituted xylan polymers (Table 2-2), which can be fractionated and extracted as soluble dietary fibres, or XOS, with varying degrees of polymerisation, through hydrothermal treatments (Reis *et al.*, 2014, 2015; Severini *et al.*, 2015; Coelho *et al.*, 2016; Lynch, Steffen and Arendt, 2016). These XOS dietary fibres are also slightly sweet, with low glycaemic response (GI index <10), and can therefore be used as sweeteners in food formulations (Mitchell, no date; Vazquez *et al.*, 2000; Carvalho *et al.*, 2013; Chemin *et al.*, 2015). XOS represents useful ingredients for food formulations, due to their range of technological properties, its lack of off-taste, and greater stability over a wide range of pH and temperatures, compared to fructo- (FOS) and galacto-oligosaccharides (GOS) (Vazquez *et al.*, 2000; Fallourd and Viscione, 2009).

XOS are valued for their reported beneficial health effects, in particular, the prebiotic effects that can be as effective as the more commonly known fructo-oligosaccharides and inulin (Vazquez *et al.*, 2000; Moura 1 , Carvalheiro 2 , Esteves 3, 2008; Aachary and Prapulla, 2011;

Carvalho *et al.*, 2013; Gomez *et al.*, 2015). The degree of polymerisation (DP) of XOS is reported to be an important factor affecting the biological properties of XOS, with short-chain XOS (DP<10) having a greater bifidogenic or prebiotic effect (Moura, Carvalheiro, Esteves, 2008). Short-chain XOS and XOS substituted with arabinose, known as arabino-xylo-oligosaccharides (AXOS), have been found to be highly fermentable *in vitro* by beneficial Bifidobacterium of the human gut (Gomez *et al.*, 2015; Sajib *et al.*, 2018). Therefore XOS product quality as prebiotic is related to the level of substitution with arabinan and degree of polymerisation, as well as it purity. Applications of XOS are found in functional foods products as low-calorie sweeteners, prebiotics and texturisers, and are also marketed as supplements (Brink, no date; Nv, no date; NutraSource, 2013; Fărcaş, 2014; Rastall and Gibson, 2015; Steiner, Procopio and Becker, 2015; Collins and Reid, 2016).

Market reports suggest that prebiotics are valued at >US$5 000 per tonne, and that the market for prebiotics will grow by a compound annual growth rate (CAGR) +10.4%, to US$7.5 billion by 2024 (Llzo and Lx, no date). Additionally, various studies suggest that the possible application of XOS in manufacturing of biomass-derived polymers, nanocomposites, gels and films will increase demand for XOS in the near future (Vallejos *et al.*, 2012; Stepan, 2013; Wang, 2014; Ren *et al.*, 2015; Heikkinen, 2016; Hettrich *et al.*, 2017; Naidu, Hlangothi and John, 2018). Hydrothermal processing technologies making use of LHW treatments are reported to obtain these XOS from BSG (Gomez *et al.*, 2015; Sajib *et al.*, 2018).

2.3.1.2 Xylitol sugar replacer

Various reports have shown that BSG hemicelluloses can be extracted and hydrolysed to produce monomeric xylose sugars, which can be readily fermented into xylitol. Such BSG-derived xylose hydrolysates are reported to have a distinct advantage in xylitol production through fermentation, compared to xyloses from other lignocellulosic materials, since BSG hydrolysates require no nutrient supplementation or detoxification to be suitable for xylitol production by yeasts (Carvalheiro, Duarte et al., 2005; Duarte et al., 2004; Mussatto et al., 2013; Mussatto and Roberto, 2005). Xylitol is a high-value bulk commodity chemical, with established markets and a market price of US$4 500 per tonne; it is the highest-value chemical of 32 identified biomass-derived chemicals with future high-potential markets (Biddy *et al.*, 2016). It is expected that the market for xylitol will grow by CAGR +6.5% in value, to over US$1 billion by 2020 (De Albuquerque *et al.*, 2014).

Xylitol is a naturally occurring sugar alcohol, part of the group polyol family; it is a unique sweetener because it is made from pentose sugar and has about a third fewer calories for the equivalent sweetness of sucrose (Rafiqul and Sakinah, 2013). Discovered in 1891, xylitol has been used as a sweetening agent in foods since the 1960s (Mitchell, no date). Xylitol is safe for diabetics since its metabolism is independent of insulin and its use is reported to prevent

tooth decay and ear infections. Xylitol is used in foods such as chewing gum, gum drops and hard candy, and in pharmaceuticals and oral health products, such as throat pastilles, cough syrups, chewable multivitamins, toothpastes and mouthwashes (Ur-Rehman *et al.*, 2013). Current commercial xylitol production is done through the catalytic hydrogenation of xylose that is obtained from the acid hydrolysis of xylan hemicelluloses in lignocellulosic biomasses, including corncobs and birch wood (Venkateswar Rao *et al.*, 2015; Ribeiro *et al.*, 2016; Özüdoğru, 2018). BSG is an excellent source biomass, and has a hemicelluloses content that is high in xylan, which can be used to produce xylose and XOS through hydrothermal treatment technologies, including Liquid Hot Water (LHW) and steam explosion (SE).

2.4 Fractionation of BSG

2.4.1 Dewatering and separation of proteins and starches from BSG by mechanical treatment

Since BSG consists of 70-85% water, it contains a substantial amount of water-soluble materials, which can make up 20-27% of the BSG dry mass; these materials include salts or minerals, sugars, starches, proteins and carbohydrates (Beldman, Hennekam and Voragen, 1987; Qin, Johansen and Mussatto, 2018). Screw type presses for biomass dewatering applications are reported to be effective and mechanically simple, and can operate continuously and unsupervised, unlike other mechanical devices, such as filter presses, belt presses or centrifuges (Williams, no date; Vavpot, Williams and Williams, 2014).

2.4.1.1 Screw press fractionation and dewatering of BSG

Screw press drying of BSG, using a single screw or twin screws, is industrially well established, and BSG moisture content can be reduced to close to 60 wt.% (Huige, 1994; Menukhov, 2006; Lopez, Pontiggia and Fernandez, 2010; Scheller and Michel, 2012; Weger *et al.*, 2017). Industrially, up to around 67 wt.% moisture content is commonly achieved with a screw press, and the pressed dried product is commercially known as brewers' pressed grains (Huige, 1994). Applying such dewatering with a screw press (>60% reduction in moisture) can reduce energy requirements in BSG thermal drying systems significantly (Weger *et al.*, 2017).

2.4.1.2 BSG screw press liquid fraction

Mechanical dewatering with a screw press can remove between 50 and 75% of the water fraction from BSG, including soluble and insoluble materials, to create a liquid product stream of between 400 and 700 kg/ton BSG processed (Finley, Walker and Hautala, 1976; Weger *et al.*, 2014; Weber and Stadlbauer, 2017). A typical liquid fraction obtained from screw press processing of BSG is reported to contain about 3.9% soluble matter, which consists primarily

of soluble materials sugars (65%), and insoluble matter, such as 13% crude protein, 11% fibre, 4% fats and 8% minerals content (Jovanka Levic, Slavica Sredanovic, 2006). Thus, this liquid product, in itself, could be refined into high-value food products.

A few alternative uses for the substantial liquid fraction obtained from BSG dewatering have been reported. Some studies described the BSG press liquid fraction as an excellent feed for methane production through anaerobic digestion, with an organic degradation rate of up to 90% and a specific methane production of 0.725 m^3/kg dry mass and 45% more gas than for raw BSG anaerobic fermentation (Weber and Stadlbauer, 2017; Weger *et al.*, 2017). In another application, from this press liquid, the separation of an insoluble fraction by filtration or centrifugation is proposed; the dried insoluble fraction, high in BSG protein (>50% crude protein), is called brewers' dried protein (BDP) (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Schwencke, 2006). The remaining clear liquid is reported to be either simply be discarded (Schwencke, 2006; Lopez, Pontiggia and Fernandez, 2010) or recycled back into the brewing process (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Huige, 1994). In the United States, this clear liquid is reportedly concentrated through multiple effect evaporators to 60 wt.% dry mass, and sold as feed binder, referred to as brewers' condensed solubles (BCS). Suspended solids are usually reduced to under 0.5% by centrifugation, while celullase enzymes can be used to lower the viscosity of BCS. BCS is highly susceptible to unwanted microbial spoiling, and preservatives or antimicrobial agents must be used to control this tendency (Huige, 1994).

2.4.1.3 Structural and chemical effects of mechanical treatment on BSG by a screw press

Screw pressing for dewatering biomass will apply different types of mechanical forces, including shear, compression and friction, which can cause disruption of biomass structures and reduce particle sizes. These effects can alter biomass properties, such as bulk density or surface-to-volume ratio, which may improve accessibility for reagents present in hydrothermal processing (Yan and Modigell, 2012; Barakat *et al.*, 2014; Yan *et al.*, 2014). Moreover, screw press dewatering BSG will selectively remove a large portion of the fine particles suspended in the press liquid with the typical (approx. 0.6 mm) screen used (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Huige, 1994). This reduction in fine particles and reduction in water can increase biomass bed porosity (Brownell and Saddler, 1986; Sui and Chen, 2015). For example, increased porosity of biomass can improve steam penetration for more efficient and fast heating in low-moisture hydrothermal treatments that use direct steam heating (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Sui and Chen, 2015).

Additionally, the large fraction of water removed in the dewatering step will also selectively remove a large portion of salts, ash, starches and proteins present in wet BSG that can affect

subsequent hydrothermal treatment. Reports using other biomass showed a reduction of these components resulted in reduced buffering leading to higher reaction rates and acidification during hydrothermal treatments (Jacobsen and Wyman, 2000; Liao *et al.*, 2004). Therefore, screw press dewatering of BSG can affect subsequent hydrothermal treatment of BSG significantly, although hydrothermal processing of screw-pressed BSG has not been reported.

2.4.2 Fractionation of BSG by hydrothermal treatment

To enable targeted valorisation of BSG hemicellulose into products, including XOS and xylose, efficient separation of components of BSG is required. This separation can be achieved through hydrothermal treatments (HTT) that disrupt the lignocellulosic structure and selectively solubilise the hemicellulose polymers, while retaining the cellulose and lignin as an insoluble residue (Hettrich et al., 2017; Naidu et al., 2018; Negahdar et al., 2016). HTT is the most widely researched and applied technology for lignocellulosic biomass deconstruction, fractionation and conversion into constituent sugars and other chemical components (Wyman et al., 2005). HTT is classified as physiochemical treatment method that mainly relies on the hydrolytic action of [H+] ions in liquid water or saturated steam at temperatures between 120-250 °C (Bayer, 2007; Ibbett *et al.*, 2011; Kruse and Dahmen, 2015). In HTT, water plays multiple roles: (i) as heat transfer medium, (ii) it physically mobilize the polymers in biomass cell walls, (iii) as a co-reactant in activated intermediates and, (iv) is a solvent for reactants and products (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Yang *et al.*, 2004; Ibbett *et al.*, 2011). Temperature and time are the main process variables considered that define mainly HTT reactions, while variations in pH are known to affect the process significantly (Figure 2-2). HTT reactions, mostly endothermic, are considered generally defined to occur in processes up to temperatures of 250 ºC, although more exothermic hydrothermal carbonisation reactions can start to occur at temperatures above 200 ºC. Water addition to biomass up to *circa* 20 wt. % significantly increased exothermic nature of the hydrothermal reactions above 200 ºC (Ibbett *et al.*, 2011).

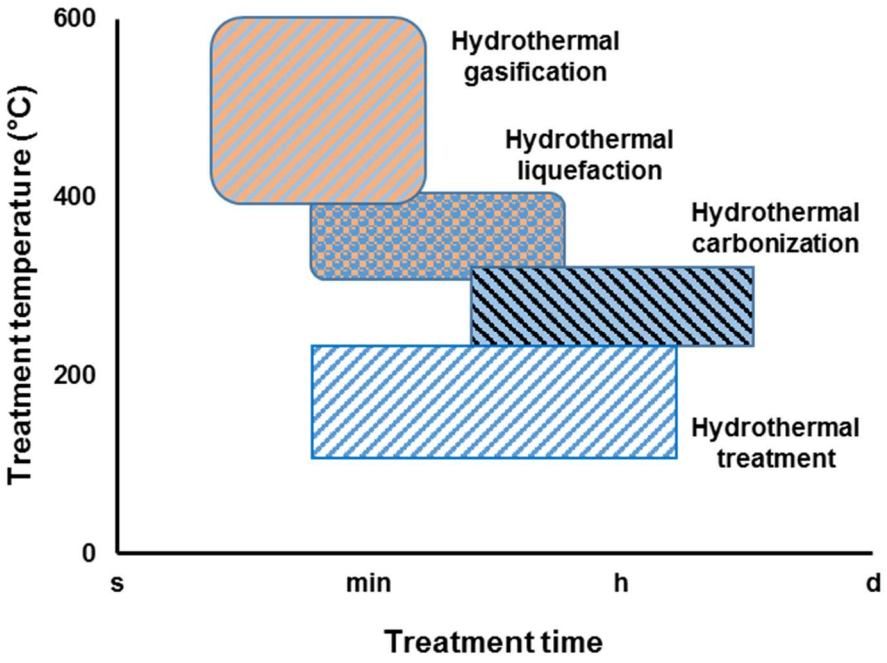


Figure 2-2 Process conditions of water thermal treatments (redrawn Matsumura, (2015))

HTT process has been applied in various reactor systems such as stirred batch vessel systems (Weil *et al.*, 1998; Vegas *et al.*, 2005; Gomez *et al.*, 2015), stationary flow-through systems (Yang *et al.*, 2004; Torres-Mayanga *et al.*, 2019) or in continuous mode with screw systems (Heitz *et al.*, 1991; Monschein and Nidetzky, 2016), plug flow systems (Thompson and Grethlein, 1979; Church and Wooldridge, 1981; Brennan *et al.*, 1986), while using direct steam injection, conductive or microwave heating (Roos *et al.*, 2009). The process can be conducted with water mainly the liquid phase, such as in stirred (Gomez *et al.*, 2015) or unstirred batch vessels (Vallejos *et al.*, 2012), or in single phase steam (Stephen Glen Allen *et al.*, 2001), or in mixed liquid water and vapour phase such as in steam explosion (Brownell and Saddler, 1987; Bayer, 2007; Roos *et al.*, 2009; Lischeske *et al.*, 2016). Various technical terms are reported to describe these HTT process configurations and their applications, such as liquid hot water (LHW), hot water, pressure cooking by water, compressed hot water, autolysis, autohydrolysis, autohydrolytic treatment, autocatalysis, hydrolytic processing, hydrothermolysis, water pre-hydrolysis, pre-treatment, steam treatment, steam-aqueous treatment and steam gun/explosion (Garrote, Domínguez and Parajó, 2002; Bayer, 2007).

Solubilisation of BSG hemicellulose through targeted HTT will selectively depolymerise the xylan polymer into XOS, shorter-chain oligomers, xylotriose, xylobiose and monomeric xylose (Kabel *et al.*, 2002; Carvalheiro *et al.*, 2005b; Shen and Wyman, 2011; Cardenas-toro *et al.*, 2014; Aguedo, Ruiz and Richel, 2015; Gallina *et al.*, 2018; Steiner *et al.*, 2018). The two main industrial HTT technologies considered for fractionation of BSG are LHW and steam explosion. Both technologies can be operated autocatalytically or with extra added catalyst.

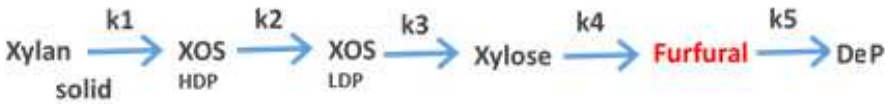
2.4.2.1 Hydrothermal treatment with no added catalyst

Autocatalytic HTT is the HTT processing that do not use any added reagents, and at mild process conditions (150 to 210°C), these treatments can selectively solubilise the hemicellulose from BSG or several other types of lignocelluloses as XOS, while the cellulose and lignin components are retained in the solid residues (Kabel *et al.*, 2002; Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015; Steiner *et al.*, 2018). Temperatures and residence times have significant effects on the XOS side chain constituents, yield of polymers, and the distribution of the degree of polymerisation (Carvalheiro *et al.*, 2004; Coelho *et al.*, 2014).

In autocatalytic HTT, the pH is not controlled, but is acidic, due to the weak acid properties of water at elevated temperatures. Additionally, the pH is further lowered with formation and release of acetic, furanic and phenolic acids during solubilisation of components of BSG and depolymerisation of hemicellulose (Pedersen and Meyer, 2010; Pedersen, Johansen and Meyer, 2011). In autocatalytic HTT, glycosidic bonds in the xylan polymer are cleaved through autocatalytic hydrolysis by H+ ions of water (Garrote, Domı and Parajo, 2004; Agbor *et al.*, 2011).The hydrolysis reaction for cleaving the xylan polymer glycosidic bonds is represented in Figure 2-3.

Figure 2-3 Representation of hydrolysis reaction

First-order kinetic models are used to describe the autocatalytic HTT between 150 and 190 °C for the solubilisation and depolymerisation of xylan polymers from BSG hemicellulose. It is proposed in the model that xylan polymers from BSG hemicellulose, first, converts into XOS with a high degree of polymerisation, which then decomposes into XOS with a lower degree of polymerisation (Carvalheiro *et al.*, 2005a). The XOS with a lower degree of polymerisation are then hydrolysed further, to xylose monomers (Carvalheiro *et al.*, 2005a). Degradation products are produced from furfural, the dehydration product from xylose (Figure 2-4).



HDP = high degree of polymerisation; LDP = lower degree of polymerisation; DeP = degradation products

Figure 2-4 Kinetic model for xylan autocatalytic depolymerisation and degradation with first-order rate coeficients k1-k5

XOS quality is informed by the degree of polymerisation and degree of XOS substitution, primarily with arabinose (Moura, Carvalheiro, Esteves, 2008; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015; Martinez-Hernandez, Tibessart and Campbell, 2018;

Amorim, Silvério and Rodrigues, 2019). The impact of temperature on BSG xylan solubilisation, products and furfural formed in HTT was discussed by inference to the reaction rate coefficients obtained for various reaction steps in the autocatalytic HTT hydrolysis (Carvalheiro *et al.*, 2005a). It was shown that increasing temperature had increased the rate at which xylan solubilises to XOS, and the rate of XOS depolymerisation, in a similar proportion. However, the rate at which xylose degrades to furfural with increasing temperature had increased faster than the rates at which XOS depolymerisation and xylan degradation increased with temperature. This shows that xylan degradation to furfural had a higher sensitivity to increased temperatures relative to hemicellulose xylan solubilisation to XOS. This is in agreement with reports that lower temperatures in hemicellulose HTT could reduce degradation by-product formation (Kupiainen, Ahola and Tanskanen, 2014).

A 'severity factor" (R_o) reportedly developed by Overend et al., (1987) combines effects of temperature and reaction time in HTT to control treatment operation and while allowing for comparison between HTT treatments (Kabel *et al.*, 2007; Stelte, 2013; Lischeske *et al.*, 2016). For a broader application, the severity factor was adapted to incorporate the impact of the pH in treatments. The effect of final pH, together with treatment time (t) and temperature (T) is described by a combined severity factor (CSF) or the log (R'_0) as (Chum *et al.*, 1990; Pedersen and Meyer, 2010),

$$\log R'_0 = \log (t.\exp^{((T-100)/14.75)}) - pH. \qquad \text{(Eq 2.1)}$$

Thus, while increasing the severity generally leads to an increase in the amount of xylan solubilised, the amount of XOS in the final product would generally decrease, while the monomer xylose and degradation products increase.

2.4.2.2 Hydrothermal treatment with extra catalyst added

An important parameter in HTT is the pH of the process, which affects the hydrolysis process (Weil *et al.*, 1998; Mosier, Ladisch and Ladisch, 2002; Mosier *et al.*, 2005; Li *et al.*, 2014). In autocatalytic HTT, the pH in the hydrolysate is not controlled or adjusted (Mosier *et al.*, 2005). However, catalysed HTT can include preloading biomass feedstock or the water with an extra acidic (SO_2, CO_2 or H_2SO_4) or alkali (NaOH or Na_2CO_3) catalyst to effectively adjust the pH in the process (García-Aparicio *et al.*, 2011; Gurgel, Pimenta and Curvelo, 2014; Li *et al.*, 2014). The addition of an acidifying catalyst directly manipulates the H+ concentration, and reduces the activation energies of the various reaction steps during HTT (Mosier, Ladisch and Ladisch, 2002). The resulting effect of extra catalyst addition in HTT can lead to higher rates of solubilisation, depolymerisation, xylose and degradation product formation, at reduced temperatures. There is an additional trade-off from extra catalyst addition in HTT, such as

LHW and steam explosion, since the additional chemicals added in the process have a cost and environmental burden (Eggeman and Elander, 2005; Kumar *et al.*, 2009; Galbe and Zacchi, 2012). Adding acid also brings the risk that extracted hemicellulose can be in the form of xylose monomers, rather than XOS. Therefore, balance is required between the amount of catalyst and the desired product, xylose or XOS. Most HTT reported used additional acid catalyst, with a focus on optimising monomeric sugar production (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005).

2.4.2.3 Application of hydrothermal treatments to BSG

a) Liquid hot water treatment

LHW involves the HTT processing of biomass in compressed (subcooled) water at temperatures between 150 and 210 °C, generally without an additional catalyst, autocatalytically. The advantage of LHW autocatalytic HTT is that mainly oligosaccharides can be produced from hemicellulose, with minimal formation of monomeric sugars (Laser *et al.*, 2002; Wu *et al.*, 2008; Kim *et al.*, 2009; Yang and Wyman, 2009). This is of particular value for scenarios where XOS is the product of interest. LHW treatment of BSG is reported for selective hemicellulose solubilisation from BSG (Kabel *et al.*, 2002; Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015). A summary of the important reports on autocatalytic LHW XOS production from BSG is given in Table 2-4.

Table 2-4 Autocatalytic liquid hot water HTT of BSG XOS production

Feedstock preparation	Composition	Solids loading	Autocatalytic HTT conditions	XOS/ ArOS[a] Yield	Reference
BSG / Dried at 50 ºC	Xylan 20.6% Arabinan 9.0% Protein 24.6%	11% dm	190 ºC 5 min	61% XOS	Carvalheiro et al., (2004b)
Barley husk air-dried, milled	Xylan 26.8% Arabinan 5.7% Protein 5.9%	11% dm	nonisothermal 200 ºC/ total 40 min	67% XOS	Garrote et al., (2004)
Mixture BSG and barley husk / Dried	Xylan 25.9% Arabinan 3.9% Protein 5.9%	11% dm	nonisothermal 207 ºC	66.4% XOS	Vegas et al., (2005)
Mixture BSG and barley husk / Dried, destarched at 130 ºC	Xylan 23.2% Arabinan 6.4% Protein 7.7%	11% dm	185 ºC 22 min	74.8% XOS	Gullón et al., (2011)
BSG / Dried at 25 ºC, homogenised, destarched at 100 ºC 30 min	Xylan 17.4% Arabinan 8.0% Protein 24.1%	11% dm	nonisothermal 200 ºC	77% XOS /20% ArOS	Gómez et al., (2015)
			nonisothermal 195 ºC (SF 3.65)	70% XOS /36% ArOS	
			isothermal 180 ºC 12.2 min (SF 3.65)	67% XOS /45% ArOS	
BSG / 40 °C 12h, dried, milled sieved 1 to 5 mm	Xylan 16.7% Arabinan 8.8%	9% dm	190 ºC 30 min (SF 4.13)	60.5% XOS (64.1% Xylan)	Michelin and Teixeira, (2016)
BSG / Dried at 60 °C	Xylan 12.5% Arabinan 5.3%	17-20% dm	160 ºC 5 min	18-23% XOS	Pinheiro et al., (2019)

BSG / Washed, 50 °C, dried, milled <1mm Microwave heated	Xylan 20.7% Arabinan 8.0%	9% dm	192.7 ºC 5.4 min	60% XOS	López-Linares et al., (2019)
a Arabinan/arabinose oligomeric					

All the reported LHW HTT studies on XOS production from BSG used 9-11% dry matter (Table 2-4) and none used a screw press dewatering step. Some did prewash the BSG at elevated temperatures before oven-drying the sample again for use (Table 2-4), although this is not an industrially-relevant approach. The reported maximum yields for XOS production from BSG in LHW treatment using non-isothermal treatments achieved up to 77% XOS yields (Gomez *et al.*, 2015), while XOS yields ranged between 61 and 74.8% in isothermal operation reported from BSG with 9-11% dry matter content (Carvalheiro *et al.*, 2004; Vegas *et al.*, 2005; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). Yet XOS highly substituted with arabinose is the most desirable, which was achieved by increasing the processing temperatures from 140 to 180 °C; however, with a further increase (>180°C), the ratio of arabinose to xylan (A/X) decreased (Garrote, Domı and Parajo, 2004; Coelho *et al.*, 2014). Therefore, instead of applying 195 °C non-isothermally (optimal condition for maximum XOS+ArOS), reducing the temperature, by using 180 °C, yet with t = 12.2 min (equal severity factor of 3.65 as optimal HTT condition) resulted in comparable XOS yields (Table 2-4), yet with higher arabinan (ArOS) recovery (Gomez *et al.*, 2015). The XOS obtained at reduced temperatures would be preferred since it results in more XOS substituted with arabinose however also leads to XOS with longer xylan chains At process conditions of 190 °C and 5 min for the maximum XOS yield of 61%, up to 39% of the XOS product had a DP>9, while only 16.7% consisted of the desired short-chain xylobiose and xylotriose oligosaccharides (Carvalheiro *et al.*, 2004). Proteinaceous compounds were reported to make up the largest fraction (>30%) of non-volatile impurities in the hydrolysate liquor. Subsequent purification of the oligosaccharide liquor was achieved by diafiltration and ion exchange, to yield a XOS product with 84 wt.% oligosaccharide content (Gomez *et al.*, 2015). Interestingly, two different XOS yield optimisation studies using treatment time and temperatures from Carvalheiro et al., (2004b) and López-Linares et al., (2019) showed in Table 2-4 found similar optimal process condition for XOS production (190 ºC/5 min and 192.7 ºC/5.4 min) and with comparable yields (61% and 60% XOS yield), suggesting low sensitivity of XOS yields in HTT to the BSG preparation and composition.

b) Extra catalyst addition in LHW hydrothermal treatment

In literature, BSG hemicellulose solubilisation and extraction are reported through HTT with dilute acid conditions or acid loadings in excess of 20 mg/g dry BSG (>0.7 wt.% acid concentrations). More recently, reductions of acid catalyst additions have been reported, as

researchers aim to minimise negative impacts and cost of adding catalysts (Table 2-5). The first report on an optimisation of BSG hemicellulose solubilisation used 240 mg acid/g dry BSG at 130 °C (Carvalheiro *et al.*, 2004). The acid dosage was lowered to 100 mg acid/g dry BSG at 121 °C and 17 min that recovered >90% of BSG hemicellulose and >85% xylan in the hydrolysate (Mussatto and Roberto, 2005). White et al. (2008) investigated BSG hemicellulose solubilisation using 50 mg acid/g dry BSG around 121 °C and, more recently, Wilkinson et al., (2014) applied acid loadings of between 20 and 40 mg acid/g BSG; however, higher processing temperatures of between 140 and 160 °C were used with 26% dry matter content BSG. Although SO_2 catalyst use is not reported for LHW of BSG, reports using other biomass show similar high xylose yields can be achieved compared to H_2SO_4 (Fan *et al.*, 2014). Yet to the author's knowledge the use of even lower acid loadings, though <20 mg/g dry BSG in LHW treatments have not been reported for BSG hemicellulose solubilisation and xylose production (Table 2-5).

Table 2-5 Acid catalysed LHW in hemicellulose solubilisation

Feedstock preparation	Solids loading	Hydrothermal conditions	Hemicellulose yield	Reference
BSG / Washed 1 h at 100°C, dried (>0.5 mm retained)	11% dm	130 °C 15 min 240 mg H_2SO_4	95% xylose	Carvalheiro et al., (2004a)
BSG / Washed 1 h at 100°C, dried, homogenized (autocatalytic/ APH)	11% dm	190 °C 2.5 min/APH 2wt.% H_2SO_4 121°C 15 min (CSF 1.10)	>98% xylose from oligomers	Duarte et al., (2004)
BSG / Washed, dried	11% dm	121 °C 17 min 100 mg H_2SO_4	85.8% xylose	S. I. Mussatto and Roberto, (2005)
BSG / Washed, dried	9% dm	120 °C 27 min 120 mg H_2SO_4	66.9% xylose	Mussatto and Roberto, (2006)
BSG / Dried, milled	17% dm	121 °C 15 min 50 mg HNO_3	37% xylose	White et al., (2008)
BSG / Oven dried 105°C, milled <212 µm	20% dm	121 °C 30 min 40 mg HCl	43% xylose	Wilkinson et al. (2015)
BSG / Washed, dried	11% dm	155 °C 0 min 160 mg H_3PO_4	88% xylose	Rojas-Chamorro et al., (2018)
Corncob	9% dm	142 °C 98 min 216 mg SO_2	90.5% xylose	Fan et al., (2014)
APH – Acid posthydrolysis				

These low catalyst additions for LHW treatments, of less than 20 mg/g dry lignocellulose (<0.7 wt.% acid concentrations), are referred to as extremely low acid (ELA) dosing LHW HTT (Liu and Wyman, 2004; Jensen *et al.*, 2010; Shen and Wyman, 2011; Gurgel *et al.*, 2012; Kim, Ryu and Oh, 2016). Nevertheless, added catalyst dosages of as low as ~5 mg/g dry matter biomass have been recommended for solubilising hemicellulose in other types of lignocellulose; it is reported to offer distinct advantages over LHW, such as a possible reduction in operating temperatures and increased hemicellulose product recovery (Yang and Wyman, 2009; Hongbin *et al.*, 2014; Martínez-Patiño *et al.*, 2015). However the acid

neutralising effect of lignocelluloses is especially marked at such low acid dosages and more importantly so when applying high solids loadings processing (Jacobsen and Wyman, 2000; Liu and Wyman, 2004). A 0.2 wt. % H_2SO_4 dosage was found to be close to 70% neutralised at high solids loadings HTT (Cahela, Lee and Chambers, 1983); and Morinelly et al., (2009) found while using 0.25 wt.% H_2SO_4, that a doubling of solids loading reduced the rate of depolymerisation of XOS to xylose by two thirds. Therefore solids loading is an important factor to consider when applying ELA dosing in LHW HTT. Nevertheless, since there are major differences in how different types of lignocelluloses react under a specific set of HTT conditions, it is impossible to say that ELA HTT results will be similar when applied to another lignocellulose such as BSG.

Moreover, adding extra acid catalyst can cause negative process requirements and have environmental impacts, as neutralisation of the residual spent acid in the hydrolysate produces by-products, such as gypsum. Yet, at low ELA dosages, <20 mg/g biomass, the addition of acid in LHW HTT has a minimal impact on the environment – similar to using no acid at all (Liu and Wyman, 2004; Jensen *et al.*, 2010; Shen and Wyman, 2011; Gurgel *et al.*, 2012; Kim, Ryu and Oh, 2016). Therefore, utilising ELA dosing in the LHW HTT process of BSG must be considered for the possible improvement of hemicellulose solubilisation and XOS production while carefully considering the effect of dry matter content.

c) Steam explosion

Steam explosion (SE) is an HTT technology where direct steam injection is used to rapidly heat biomass under high-pressure, and it is appropriate for high dry matter content biomass treatment (>25 wt.% dry matter) (Chen, 2015; Kokta and Ahmed, 1998; Cullis, Saddler and Mansfield, 2004; Energy, 2015; Jacquet *et al.*, 2015; Monschein and Nidetzky, 2016). Steam explosion process conditions can range between 150 and 235 °C, with residence times from a few seconds to 30 min (Chen, 2015; Stelte, 2013). At the at the end of the reaction time, the biomass is expelled with explosive decompression (rapid depressurisation) into a flash tank (Chen, 2015; García-Aparicio *et al.*, 2011; Yang and Tucker, 2013), which rapidly cools the reaction slurry and aids mechanical biomass deconstruction (Chen, 2015; Brownell and Saddler, 1987). Although only a few batch steam explosion systems have the capability of mechanical mixing (Liao *et al.*, 2016), continuous screw type systems have improved liquid-solid mixing and flow characteristics (Richard *et al.*, 1990; Heitz *et al.*, 1991; Stelte, 2013; Chen, 2015; Borén *et al.*, 2018).

Various studies report using steam explosion for XOS production from lignocellulosic biomass, such as bagasse (Carvalho *et al.*, 2018), barley husk (Persson, Dinh and Jönsson, 2009; Roos *et al.*, 2009) and corncobs (Teng *et al.*, 2010). XOS yields of up to 25% have been reported

for steam explosion using barley husk, and 41.3% from corncobs hemicellulose (Table 2-6). Previous steam explosion studies on BSG focused primarily on total sugar production (Shindo and Tachibana, 2004, 2006) and enzymatic digestibility of the cellulose in solid residues after the process (Qihua *et al.*, 2010; Pierre *et al.*, 2011; Kemppainen *et al.*, 2016), rather than aiming to maximise hemicellulose solubilisation and recovery in the hydrolysate. For example, when steam explosion on BSG took place at very high temperatures and dry matter (235 °C, 21.5 wt.% dry matter), up to 24.6% of the dry mass was recovered in the hydrolysate as a mixture of C5 and C6 sugar (Shindo and Tachibana, 2004), indicating significant hydrolysis of both hemicelluloses and celluloses had occurred yet not selective for the XOS or xylose. A few studies report the removal of hemicellulose from BSG, which can be used as an indicator of the suitability of the steam explosion conditions for possible recovery of XOS and/or xylose from BSG xylan (Table 2-6).

Table 2-6 Steam explosion in XOS production

Feedstock preparation	Starting solids loading	Steam explosion conditions	XOS yield	Reference
BSG / Wet raw	21.5% dm	235 °C 1 min	24.6% dm sugars	Shindo and Tachibana, (2004)
BSG / Oven dried	92% dm	180 °C 1 min	>35% hemicellulose removed	Qihua et al., (2010)
BSG / Wet raw, frozen	30.8% dm	180 °C 10 min	<20% hemicellulose removed	Kemppainen et al., (2016)
		200 °C 10 min	~16% xylan yield (>70% xylan removed)	Rommi et al., (2018)
BSG / Wet raw, frozen	23.9% dm	200 °C 10 min	17.6% xylan yield (37.1% xylan removed)	Rojas Pérez, (2018)
BSG / Alcalase deproteinised	23.9% dm final	180 °C 30 min	22% xylan yield (29.2% xylan removed)	Rojas Pérez, (2018)
Barley husk	30% dm	210 °C 5 min	16% XOS yield	Roos et al., (2009)
Barley husk	25% dm	200 °C 10 min	25% XOS yield	Persson et al., (2009)
Corncob	33% dm	180 °C 10 min	41.3% XOS yield	Wang et al., (2013)

Comparing the conditions of steam explosion fractionation, residues show large variations in hemicellulose removal from BSG (Table 2-6). Steam explosion of raw BSG from a brewery with 31 wt.% dry matter, for 10 min, achieved a >70% reduction in hemicellulose at 200 °C (Rommi *et al.*, 2018), while at 180 °C, less than 20% was removed from the same BSG (Kemppainen *et al.*, 2016). In a study by Qihua et al. (2010), however, more than 35% hemicellulose was removed by steam explosion at 180 °C after only 1 min using 92 wt.% dry matter BSG (oven dried, not screw pressed). This variation in results reported supports the need to conduct a more detailed investigation of xylan solubilisation and XOS yield from BSG by steam explosion, to improve understanding of the relationships between treatment times, temperature and dry matter content used in the process

d) Extra catalyst addition in steam explosion hydrothermal treatment

Steam explosion, as a HTT technology (Brownell and Saddler, 1987; Bayer, 2007; Roos *et al.*, 2009; Lischeske *et al.*, 2016), follows similar xylan solubilisation and XOS depolymerisation hydrolysis kinetics as LHW, therefore, the addition of extra acidifying catalyst can provide similar benefits as described above, including increased hemicellulose solubilisation and lower treatment temperatures (Tengborg *et al.*, 1998; Öhgren, Galbe and Zacchi, 2005; García-Aparicio *et al.*, 2011; Diedericks, 2013; Zhang *et al.*, 2014; Carvalho *et al.*, 2018). Acid catalyst addition to the lignocellulosic biomass is reportedly done in two ways, either by direct acid loading to the biomass or in a separate soaking with dewatering step before HTT (Galbe and Zacchi, 2012; Wei *et al.*, 2018). The soaking step is an additional process step optimised for catalyst loading, soaking time (30 min-24 h) and in some cases temperature to achieve a homogeneous catalyst dispersion for optimal effect, while removing some impurities from the biomass in the spent acidic liquid. Catalysts in a gaseous form (CO_2 or SO_2) are also applied in pre-soaking step (Öhgren, Galbe and Zacchi, 2005; Carrasco *et al.*, 2010; Ewanick and Bura, 2011; García-Aparicio *et al.*, 2011), which result in reduced or no waste stream compared to dilute acids; however, the catalytic action or acidification in HTT can be less and the chemicals more expensive (Tao *et al.*, 2011).

The direct dosing of biomass is considered more efficient; the processing time, acid catalyst dosage and waste streams production, is reduced (Linde, Galbe and Zacchi, 2006; Galbe and Zacchi, 2012). Most batch steam explosion systems are not stirred vessels (Liao et al., 2016), which can result in reduced mass transfer effects that can reduce product yields, even with extra catalyst added (Chen, Wu and Lee, 1998; Lee *et al.*, 1998; Shao and Lynd, 2013). However, various continuous-flow, steam-explosion, screw-type devices are reported to enable optimised catalyst addition through improved liquid-solid mixing and flow characteristics (Humbird *et al.*, 2011; Zhang *et al.*, 2014; Ouyang *et al.*, 2015; Kapoor *et al.*, 2017). A steam explosion screw reactor using corncobs reported that almost 90% of the hemicellulose xylan was recovered as xylose when dosing involved an extra 9 mg H_2SO_4/g dry matter catalyst at 200 °C with 32% dry matter content (Zhang *et al.*, 2014), yet only batch steam explosion studies using BSG are reported (Table 2-7)..

Table 2-7 Acid catalysed steam explosion in hemicellulose solubilisation

Feedstock preparation	Solids loading	Steam explosion conditions	Hemicellulose yield	Reference
BSG / Wet raw frozen	30.8% dm	200 °C 10 min 10 mg H_2SO_4	<45% xylan	Kemppainen et al., (2016)
		180 °C 10 min 10 mg H_2SO_4	<15% xylan	
BSG / Wet raw	23.9% dm	190 °C 5 min Impregnated 12 h 0.5% H_2SO_4	30.7% xylan (0.4% loss)	Rojas Pérez, (2018)
BSG / Alcalase deproteinised	23.9% dm final	173.5 °C 15.5 min Impregnated 12 h 0.5% H_2SO_4	47.0% xylan (14.8 g total/l with 4.6 g xylose/l)	Rojas Pérez, (2018)
Bagasse	50% dm	190 °C 5 min 25 mg H_2SO_4	40% XOS	Carvalho et al., (2018)
Cornstover	20% dm	190 °C 5 min 80 mg SO_2	78% xylose	Öhgren et al., (2005)
Corncob	33% dm	200 °C 5.5 min 9 mg H_2SO_4 Continuous	90% xylose	Zhang et al., (2014)

A steam explosion investigation of BSG reports using 10 mg H_2SO_4/g BSG (30.8 wt.% dry matter) dosing, and finding that adding this extra acid at 180 °C improved hemicellulose solubilisation and recovery relative to no extra acid addition (Table 2-7); however, at 200 °C, the extra acid addition resulted in about 20% lower hemicellulose removal relative to no extra acid addition (Kemppainen *et al.*, 2016). On the other hand, Rojas Pérez, (2018) found enzymatic removal of BSG proteins before steam explosion improved xylan recovery, and showed using 0.5% H_2SO_4 catalyst resulted in maximum xylan recovery of 47.0% at 173.5 °C and 15.5 min (Table 2-7).Therefore, the steam explosion HTT work reported for BSG and XOS production is limited and is supplemented by this work.

2.4.2.4 High solids loading for intensification of hydrothermal processing

In HTT, increasing the solids content and decreasing the moisture content of lignocellulosic biomass, such as BSG, prior to HTT may increase product concentrations, reduce processing costs, because equipment with significantly smaller capacity can be used, and increased energy efficiency is possible (Larsen *et al.*, 2008; Humbird *et al.*, 2011; Leibbrandt, Knoetze and Görgens, 2011; Galbe and Zacchi, 2012; Modenbach and Nokes, 2012). Therefore as a result of a lack of applicable high solids HTT data some assume solids loading has little or no effect on lignocellulosic HTT processing and use increase solids loadings to take advantage of the benefits in process techno economic assessments (Franceschin *et al.*, 2011; Clauser *et al.*, 2015; Nieder-Heitmann, Haigh and Görgens, 2018).Yet, although HTT at higher dry matter content is desired, water plays an important role as heat transfer medium and as a solvent for reactants and products (Chen, Wu and Lee, 1998; Lee *et al.*, 1998; Shao and Lynd,

2013). Additionally water is a transporting medium for the polymers, and is co-reactant during HTT reactions (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Yang *et al.*, 2004; Ibbett *et al.*, 2011). Thus, significant negative impacts on the recovery of hemicellulose recovery with increased dry matter content of lignocellulosic biomass in HTT are commonly reported (Stephen Glen Allen *et al.*, 2001; Yang *et al.*, 2004; Ibbett *et al.*, 2011; Modenbach and Nokes, 2012), and as a result more dilute solids concentrations (<15% dry matter) are used in HTT (M. Cuevasa, M. Saleha, no date; Ewanick and Bura, 2011; Luft *et al.*, 2018).

The effect of dry matter content on hemicellulose solubilisation and xylan depolymerisation in LHW treatment of lignocellulosic biomass is not well reported. Studies show lower xylan yields are obtained from increasing dry matter content of BSG in LHW HTT (Wilkinson, Smart and Cook, 2015; Pinheiro *et al.*, 2019). Yet, some studies found that increasing solids loadings in LHW treatment of BSG increased hemicellulose solubilisation and xylan recovery (Mussatto and Roberto, 2005, 2006; Plaza *et al.*, 2017). However as previously discussed, neutralisation of acid catalyst with increased solids loadings in HTT of lignocellulosic biomass can reduce catalyst effectiveness, especially when using low acid catalyst dosing such as ELA (Cahela, Lee and Chambers, 1983; Jacobsen and Wyman, 2000; Morinelly *et al.*, 2009). When using acid dosing, Cuevas et al., (2015) found that low acid dosing (0.01M H_2SO_4 catalyst addition) with increased solids loading in HTT resulted in lower xylose yields from olive stones, while at higher acid dosing (0.03M H_2SO_4 catalyst addition), the opposite was observed. While using no added catalyst, Vallejos et al. (2012) investigated the effect of dry matter content of up to 50% dry matter in an autocatalytic HTT on hemicellulose solubilisation from bagasse. Results show positive and negative relationships with XOS yields in the process ranges investigated. Example, XOS yield increased from 34%, at 10% dry matter, to 43% at 25% dry matter at 170 °C and the same treatment time. This is in agreement with HTT studies using other xylan sources that found increased yields at higher dry matter content (Jacobsen and Wyman, 2002; Cullis, Saddler and Mansfield, 2004; Roos *et al.*, 2009).

Vallejos et al. (2012) used solids loading of up to 50% dm of bagasse in batch cylindrical reactors with no stirring, even though stirred batch systems, such as the Parr with conventional heating, are reported to be less suited to handling >15% dry matter content efficiently, due to heat and mass transfer limitations (Yang and Tucker, 2013). However, the design of steam explosion HTT systems allows for high solids (>20%) loading, since direct steam injection allows for rapid heating of biomass (Chen, 2015; Stelte, 2013; Sui and Chen, 2015). Yet generally the initial dry matter content of biomass significantly affect the heating efficiency and the resulting HTT results (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Sui and Chen, 2015; John *et al.*, 2017). In a report using steam explosion HTT (200 °C, 3 min), XOS yield from barley husk increased from 6.5 to 9.5% with increased initial dry matter of 20%

to 30% used (Roos *et al.*, 2009). However, higher dry matter content in steam explosion can also increase the apparent and combined severity of the HTT and reduce recovery of hemicellulose as a result of intensified degradation reactions (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Sui and Chen, 2015; John *et al.*, 2017). Therefore, in a HTT process for BSG hemicellulose solubilisation for XOS or xylose production, the HTT reactor vessel and the dry matter concentration of the process are important parameters for consideration, to achieve maximum overall product yield and cost efficiency.

2.4.2.5 Scale up of hydrothermal treatment technologies

Generally for lignocellulosic biomass the autocatalytic steam explosion HTT process is reported with relatively low (< 50%) hemicellulose recoveries (Chen, 2015; Laser *et al.*, 2002). LHW is reported to generally achieve better product yields than SE of up to 65% hemicellulose yields, including XOS. Acid catalyst addition to autocatalytic LHW and steam explosion HTT can increase the hemicellulose recovery, such as ELA HTT (Shen and Wyman, 2011; Gurgel *et al.*, 2012). Despite the varying results reported from the different HTT technologies, the broad hydrothermal chemistry dominating HTT for lignocellulosic biomass is the same across systems (Brownell and Saddler, 1987; Roos *et al.*, 2009; Lischeske *et al.*, 2016). Process conditions from bench scale multivariate optimisations can be used and scaled up to larger pilot scale HTT systems (generally using solids loadings in the reported suitable range for a particular system) by using the same optimal process conditions (Lischeske *et al.*, 2016), or by using similar treatments, as per severity factor (Overend, Chornet and Gascoigne, 1987; Heitz *et al.*, 1991; Roos *et al.*, 2009). However such use of severity have been shown to be less effective, compared to multivariate optimised process conditions when assessing xylan and total sugar recovery from cornstover (Lischeske *et al.*, 2016). In a comprehensive comparison of HTT in 4 reactor configurations, from 3 g batch (10% dry matter) up to 0.5 t/d (30% dry matter) continuous screw systems, reported that optimum HTT conditions identified through multi-variate optimisations from the smaller less costly batch reactors were within the range of process conditions for near-optimal yield of the largest pilot scale HTT reactor systems (Lischeske *et al.*, 2016) as shown in Figure 2-5. The near-optimal space, two standard deviations from the optimum, yields cannot be distinguished from the optimal result with a confidence of 95%.

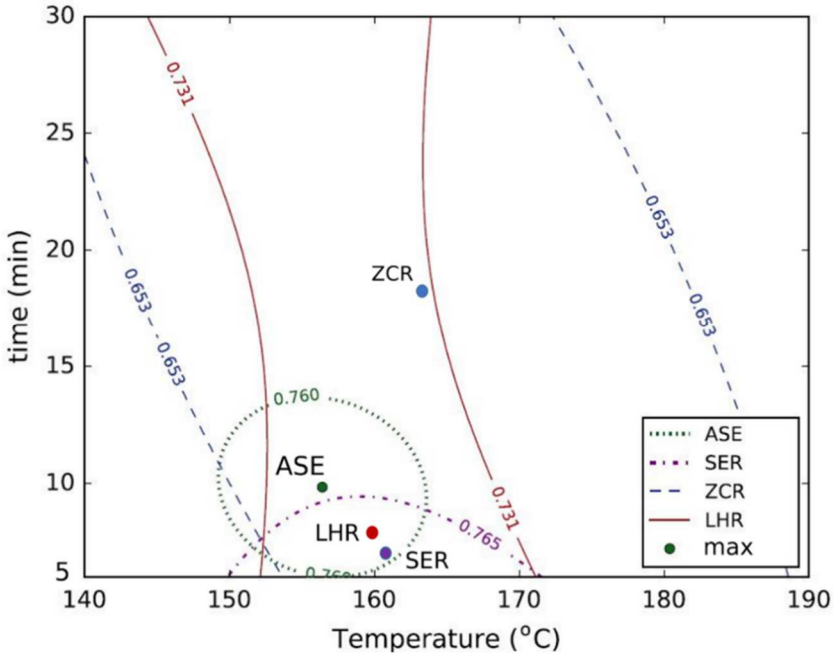


Figure 2-5 Near optimal operating space for xylan fractional yield (two standard deviations from the optimum) from cornstover using 10 mg H_2SO_4/g dm for various reactor systems: ASE- Batch flow through system solvent extraction (10% dm), ZCR 1-L stirred batch reactor Zipperclave (25% dm), SER 4-L batch SE (25-30%dm) and LHR 500 kg/d continuous screw reactor (30% dm) with optimal yields 0.789, 0.746, 0.797 and 0.766 respectively (Adapted from Lischeske et al., (2016) creative commons license)

2.4.2.6 Comparison between the hydrothermal treatment technologies

To summarise the discussion and comparison of HTT, the benefits and drawbacks of the general characteristics of the process conditions of LHW HTT, steam explosion and ELA dosing HTT, as reported by the literature, are given in Table 2-8.

Table 2-8 Comparison between different HTT processes (adapted from (Laser *et al.*, 2002; Yang and Wyman, 2009; Galbe and Zacchi, 2012; Verardi *et al.*, 2012)

Hydrothermal Treatment	Process Conditions	Advantages	Disadvantages
	LHW treatment		
Autocatalytic.	120-220 °C	No acid catalysts needed	Equipment corrosion
			Long heat up time (15-45 min)
	dry matter =<15%	High oligomer yield	High energy consumption
	2-60 min	Low inhibitors	<65% hemicellulose recovery
ELA HTT			
<0.7wt. % acid		Shorter residence time	Added acid consumption
	130-190 °C	compared to LHW with no added catalyst	compared to LHW
	dry matter =<15%	Reduced temperatures	
	2-20 min	>65% hemicellulose recovery	
	Steam explosion		
Autocatalytic	150-230 °C	Short residence time	Higher temperature
	dry matter >20%	No corrosion problem	High pressure
	5s-30 min	Fast heat up (1-5 min)and flash cooling	No stirring in batch
			<40% hemicellulose recovery

2.5 Techno-economic analysis of technologies for BSG hydrothermal processing

In this section a review is given on the general techno-economic analysis methodology, the steps involved and tools applicable to reach the objective; to evaluate and compare the process scenarios for valorisation BSG.

2.5.1 Techno-economic analysis aims and methodology

The techno-economic analysis aims to provide sufficient technical and economic analysis of the biorefinery concepts developed to understand production costs and determine profitability compared to a base case. In addition, techno-economic analysis aims to provide sufficient details in describing the technologies applied to be able to assess risks with execution in the market (Towler and Sinnott, 2008). In the following section a review of techno-economic studies on BSG valorisation with HTT are given.

2.5.2 Reported techno-economic studies on BSG valorisation concepts using hydrothermal processing technology

A survey of literature on techno-economic and life-cycle environmental impact assessments in relation to using BSG as a feedstock to produce chemical products will be discussed in the following sections. The reported studies found on BSG valorisation include products such as ethanol, xylitol, AXOS, lactic acid, activated carbon, phenolic acids and polyhydroxybutyrate.

2.5.2.1 Techno-economic evaluation for BSG valorisation in Brazil

A techno-economic evaluation in Brazil for BSG valorisation into xylitol, lactic acid, phenolic acids and activated carbon, concluded that xylitol was the most profitable product – selling at between 2.1 to 3.6 times the cost of production, depending on level of heat and mass integration applied (Mussatto *et al.*, 2013). BSG was transported at 55% moisture and the BSG was washed with water and dried to 10% moisture content for storage. The BSG was diluted again to 11% for the dilute acid HTT using 100 mg acid/g BSG at 120 °C and 17 min. A BSG cost price of US$42/t (55% moisture) was assumed that included 100 km transportation costs. However, the assumed BSG feed rate, of 100 tonne BSG per hour, is more than 450 000 tonne dry BSG per year, as much as the combined BSG produced by more than 10 large-scale breweries, or more than half the combined production of BSG in Brazil and double the total South African production (Ramukhwatho et al., 2016). Overall the heat integration strategy that resulted in a total reduction in energy needs for heating and cooling utilities, of 42.2% and 55.8% respectively. Additionally, BSG proteins were not reported to be recovered from the process.

2.5.2.2 Techno-economic evaluation for BSG valorisation in Colombia

In another techno-economic analysis of the valorisation of BSG for the production of xylitol, ethanol and polyhydroxybutyrate in Colombia found xylitol to be the most profitable product, with total cost of production reported of US$0.35/kg (A. Dávila, Rosenberg and A. Cardona, 2016). This was less than half of the xylitol production cost reported (US$0.81/kg) for the study in Brazil (Mussatto *et al.*, 2013), partly due to the reduced costs of BSG at US$ 21/t. Nevertheless, BSG feed composition, feed rate and dilute acid HTT conditions was the same in both studies. Similarly, a thermal drying step of BSG for storage was applied, where dry matter was increased from 55% to 90% dry matter. After HTT, NaOH was used for neutralising the hydrolysate to a pH of 6.5 that results in precipitation of impurities that were removed to reduce fermentation inhibitors. The hydrolysate, containing 23 g xylose/l, was concentrated by flash evaporation to 70 g xylose/l and subsequently fermented using a *Candida guilliermondii* yeast at 30 °C. In both studies xylitol crystallisation was reportedly done at 5 °C with the aid of ethanol to increase the xylitol yield. In both studies, heat integration was found to reduce heating requirements by more than 40%.

2.5.2.3 Life cycle assessment for process of AXOS production from BSG

A life-cycle assessment of a process producing ethanol and XOS from a feedstock of BSG and barley straw reports on a LHW HTT process at 210 °C with a dry matter content of ~11% (González-García, Morales and Gullón, 2018). The study considered XOS production as five production units and assessed 9 environmental impacts, acidification potential, eutrophication potential, global warming potential, ozone depletion potential, photochemical oxidation potential, human toxicity potential, freshwater aquatic ecotoxicity potential, marine aquatic ecotoxicity potential and terrestrial ecotoxicity. Results show that, in the nine global process impact categories considered, the HTT impact ranged between 33% and 55% in seven impact categories associated with the large steam requirements. Additionally, for XOS production section alone (excluding ethanol), in eight of nine environmental impacts assessed, the autocatalytic LHW treatment process contributed to more than 60% of those impacts, revealing the LHW HTT process as a hot spot. Which means this is where process intensification is required to reduce energy demands and applying high solid loadings in the process can achieve this,

2.6 Conclusions

From the literature considered a few novel areas of the work can be highlighted as follows.

2.6.1 Experimental work

Current optimised processes reported for XOS production are autocatalytic HTT processing done at high temperatures (>180 °C) and for xylose production HTT with acid catalyst (>100 mg H_2SO_4/g dm) additions at lower temperatures (<150 °C), both at low solids loadings or low dry matter (<11% dm) concentrations (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005; Gomez *et al.*, 2015; López-Linares *et al.*, 2019). Considering the reported HTT technologies, firstly, the effect of solids loading in HTT is considered followed by lowering acid catalyst addition and scale up in steam explosion HTT.

2.6.1.1 Solids loading: A process variable for optimisations in hydrothermal treatment of biomass

Both optimised processed reported for the production of XOS and xylose from BSG involved liquid hot water (LHW) HTT, in 9 and 11% dry matter concentrations (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005; Gomez *et al.*, 2015; López-Linares *et al.*, 2019). Yet the effect of solids loading in HTT on hemicellulose xylan depolymerisation as well as XOS yield is unclear and reports for other biomass show increased solids loading can lead to reduced acidification ([H+] concentration) achieved in HTT thereby reducing the rate of xylan solubilisation and lowered yields (M. Cuevasa, M. Saleha, no date; Cahela, Lee and Chambers, 1983; Jacobsen and Wyman, 2000; Morinelly *et al.*, 2009). On the other hand some HTT studies for biomass high in hemicellulose xylan showed higher solids loadings can lead to increased XOS yields (M. Cuevasa, M. Saleha, no date; Roos *et al.*, 2009; Vallejos *et al.*, 2012). From these results it is clear in HTT of biomass the initial moisture content can have a significant effect on hemicellulose solubilisation and depolymerisation results in different HTT systems. Therefore to conduct an optimisation in HTT of biomass, including BSG, the effect of the initial moisture content or solids loadings needs to be considered in addition to processing time and temperature.

2.6.1.2 Process intensification by increasing solids loading: in hydrothermal treatment of BSG

Both optimised HTT processes for the production of XOS and xylose from BSG reported were conducted in stirred batch reactors while using low (9 or 11% dm) solids loadings (Carvalheiro *et al.*, 2004; Mussatto and Roberto, 2005; Gomez *et al.*, 2015; López-Linares *et al.*, 2019). However literature shows using high (>11% dm) solids loadings in HTT biomass processing can provide a significant HTT process intensification by: (i) producing higher product concentrations, (ii) a reduction in the required process water, (ii) reduced process temperatures, and (iv) reduced heating requirements (Larsen *et al.*, 2008; Humbird *et al.*, 2011; Modenbach and Nokes, 2012). Therefore the use of high solids loadings in HTT

biomass can be advantageous for overall process economics, if the negative effects with low moisture content processing can be mitigated (Yang and Tucker, 2013). Water plays various roles in HTT processing and low moisture content can result in high viscosity, reduced mass and heat transfer, increased degradation or by-product formation and lower product yields, (Larsen *et al.*, 2008; Humbird *et al.*, 2011; Modenbach and Nokes, 2012). Steam explosion (SE) HTT technology with direct steam injection heating is more suitable for higher (>15% dm) solids loadings HTT, compared to stirred batch reactors (Yang and Tucker, 2013). However no SE study is reported for production of XOS from BSG. An optimisation for total xylan recovery (XOS and xylose combined) from a enzymatically modified BSG in steam explosion found only a 47% total xylan yield (Rojas Pérez, 2018). Reported SE studies using barely husk and corncobs show significantly lower XOS yields (<40%) can be expected, as shown in Table 2-6, compared to the bench scale HTT in dilute stirred reactors (Parr). An investigation for the production of XOS from BSG at high solids (>11% dm) during HTT is needed while assessing the effect of the moisture content on the resulting hydrolysate pH, the combined severity factor (CSF) of the HTT and acidification.

2.6.1.3 Reduced acid use by optimisation of ELA dosed hydrothermal treatment of BSG

For xylose production from BSG hemicellulose, a reduction in acid catalyst addition from reported (>100 mg H_2SO_4/g dm) dosing (Vigo-ourense and Lagoas, 2004; Mussatto and Roberto, 2005) can be achieved by using ELA dosing (<20 mg H_2SO_4/g dm) which could provide substantial process improvements considering reports on HTT from other biomass (Yang and Wyman, 2009; Hongbin *et al.*, 2014; Martínez-Patiño *et al.*, 2015). Compared with no catalyst addition, ELA additions in HTT directly increase the [H+] and rate of xylan hydrolysis that can reduce the required processing temperature (Mosier, Ladisch and Ladisch, 2002; Garrote, Domı and Parajo, 2004; Agbor *et al.*, 2011). A reduction in HTT processing temperatures can result in reduced rate of degradation by-products formation which can improve the XOS and xylose yields (Kupiainen, Ahola and Tanskanen, 2014). Additionally the beneficial effects from reduced HTT temperatures by using ELA dosing can lessen some negative effects such as increasing degradation products formation typically reported from high solids (>11% dm) loading biomass in LHW HTT (Yang and Tucker, 2013). Altogether the process intensification that can be achieved can improve the process economics of XOS and xylose production from BSG, in particular by reducing the amount of water, acid catalyst used and reducing processing temperature requirements compared to literature (Larsen *et al.*, 2008; Humbird *et al.*, 2011; Modenbach and Nokes, 2012). Therefore the relationship of ELA dosing at high solids loadings of BSG in HTT for the production of XOS and xylose needs to be investigated and optimised by minimising acid loading used while also reducing xylan degradation and fermentation inhibitors. Additionally, studies using other biomass show

benign CO_2 can be used as a catalyst in HTT instead of a corrosive mineral acid catalyst like H_2SO_4 (Gurgel, Pimenta and Curvelo, 2014). Therefore to further improve the ELA HTT process of BSG the addition of CO_2 as catalyst could be advantageous and needs investigation.

Bench scale stirred reactors are ideal for investigating ELA catalyst dosing in LHW HTT using typically H_2SO_4 and CO_2 however in SE reactor as catalyst SO_2 gas is commonly used by applying it in a pre-soaking step to the biomass. This method of applying the catalyst can lead to excess gas loss and extra process time for the pre-soaking. An alternative SO_2 dosing can be investigated by using SO_2 aqueous solution or a solid potassium metabisulfite ($K_2S_2O_5$) a SO_2 dosing agent used typically in wine industry. The effectiveness of these two SO_2 dosing methods in SE to increase the XOS yield and XOS depolymerisation to short chain XOS (xylobiose and xylotriose) must be investigated.

2.6.1.4 Hydrothermal treatment scale up by applying bench scale optimised process conditions

For XOS production from BSG, literature reports (Table 2-4) various bench scale LHW HTT optimisations of BSG with maximum XOS yields between 61-77% obtained between 190-200 ºC. Steam explosion HTT technology with direct steam injection heating is more suitable for scale up of higher (>25% dm) solids loadings HTT compared to stirred batch reactors (Yang and Tucker, 2013). However no SE study is reported for production of XOS from BSG. Reported SE studies (Table 2-6) using barely husk and corncobs show significantly lower XOS yields (<40%) can be expected compared to the bench scale HTT in dilute stirred reactors (Parr). An optimisation for total xylan recovery (XOS and xylose combined) from an enzymatically modified BSG in SE HTT found only a 47% total xylan yield (Rojas Pérez, 2018). Therefore an investigation of the HTT of BSG in SE for scale up of XOS production is required.

Literature show, using other biomass, the optimum HTT process conditions obtained from convenient bench scale LHW HTT optimisations for xylan recovery (XOS and xylose combined) can be used as near optimal process conditions for scale-up in larger HTT systems like steam explosion (Lischeske *et al.*, 2016). Perhaps an efficient method to establish a near optimum XOS yield from BSG in SE HTT at high solids loading can be found from the process conditions from reported LHW HTT optimisations for BSG conducted in smaller bench scale systems.

Different reports using LHW in similar bench scale stirred batch reactors show optimal process conditions found for maximum XOS yield from BSG were ranged between 190 ºC and 5 min to 200 ºC and 0 min (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015; López-Linares *et al.*, 2019). However to increase the overall combined yield of preferred XOS, substituted with more

temperature sensitive arabinose (ArOS), a reduced temperature of 180 ºC and longer treatment times (12.2 min) in HTT is preferred (Gomez *et al.*, 2015). This preferred process condition applies the same HTT severity factor of 3.65 obtained for combining temperature and treatment time from the optimum condition Therefore, as a method to scale up for XOS production from BSG, using the more preferred (180 ºC) and optimal (200 ºC) process conditions reported must be investigated. This method can efficiently achieve the process intensification required to improve the process economics of XOS and xylose production from BSG, in particular by reducing the amount of water used and reducing processing energy requirements. An investigation for such production of XOS from BSG at high solids (>11% dm) during SE HTT is needed while assessing the effect of the moisture content on the resulting hydrolysate pH, the combined severity factor (CSF) of the HTT and acidification.

2.6.1.5 Hydrothermal treatment technologes comparison LHW and steam explosion

Very limited studies in literature on hydrothermal treatment of biomass show comparisons between HTT tegnologies such as LHW and Steam explosion. The comparison on total xylan yield found near optimal process ranges can overlap (Lischeske *et al.*, 2016). However, the 1 L stirred reactor had the broadest near optimal process range compared to other technologies such as steam explosion. A more detailed comparison of hydrolysis results using CSF and the assessment of dry matter content on the results will give insights into the hydrothermal treatment effected achieved in each of the HTT technologies.

2.6.1.6 Efficient mechanical dewatering by screw pressing for high solids hydrothermal treatment

To achieve the reduced the moisture content of BSG in HTT processing investigations, a screw press is reported as an effective mechanical dewatering method, well proven for BSG use industrially (Huige, 1994). A screw press can be used to dewater the BSG efficiently, separate a fraction high in BSG protein while producing a dried residue with increased fibre content (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977). Such a fibre enriched BSG could be better suited for HTT production of XOS and xylose for xylitol fermentation. However, screw pressed BSG has not been reported in HTT processing investigations. Comparisons between literature suggest small variations in BSG composition and feedstock preparation may not significantly affect optimal HTT process conditions for XOS production (Carvalheiro *et al.*, 2004; López-Linares *et al.*, 2019). However, the effect of screw press dewatering through physical changes in biomass, water reduction and/or small changes by reduction in protein and ash in BSG composition can affect HTT process that could result in modification of acidification (H+ concentration) in the hydrolysate and pH. Therefore a comparison, of the acidification in the hydrolysates after HTT between pressed dried BSG and raw unpressed BSG could show possible effects in addition to water reduction.

All these factors in HTT of BSG must be assessed for effects on XOS production including XOS yields, xylobiose and xylotriose fraction in the XOS, xylose sugar yields, total dissolved solids (TDS), xylan recovery, degradation by-product formation and fermentation inhibitors.

2.6.2 Techno-economic assessment work

Survey of techno-economic studies on xylitol and XOS valorisation concepts on BSG shows while no techno-economic studies were reported for XOS production from BSG, the BSG valorisation concepts reported for production of xylitol could be economically viable (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016; González-García, Morales and Gullón, 2018).

2.6.2.1 Practical scale of BSG production

Production capacity or plant scale has a significant effect on cost of production, techno-economics and viability of the valorisation concept (K.H. Kim et al., 2001). To take advantage of cost reduction at large scale, published techno-economics for BSG process simulations used a feed rates for typical model lignocellulosic plants of more than 50 tonne dry matter per hour (Humbird *et al.*, 2011). These studies on BSG valorisation applied large scales of production *ca* 1200 tonne dry BSG per day (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016; González-García, Morales and Gullón, 2018), that are not practically viable (Buffington, 2014). A typical average medium size brewery generates *ca* 20 tonne dry per day, and only a few very large brewery units can reach *ca* 90 tonne dry BSG per day (Ishiwaki *et al.*, 2000; Ramukhwatho, Seetal and Pienaar, 2016) and a more practical scale of BSG production in techno-economic analysis is necessary, to determine if business cases for the high-value products xylitol and XOS are still feasible at reduced feed rates. The co-location with breweries of the proposed production processes should be used so to minimise transportation costs, utilities and services can be shared, and a possible reduction in capital expenditure can be achieved (Ben-Hamed, 2012; Kerby and Vriesekoop, 2017).

2.6.2.2 Process intensification by improved hydrothermal treatment technology

Additionally, the techno-economic studies reported on xylitol production from BSG applied processes that are heat intensive with the applied HTT technologies (González-García et al., 2018; Mussatto et al., 2013). Improved HTT processes of lignocellulosic biomass that use higher solids loading or dry matter can reduce the process equipment size and heat requirements significantly (Larsen *et al.*, 2008; Leibbrandt, Knoetze and Görgens, 2011; Modenbach and Nokes, 2012). Such novel processes also need to aim to reduce acid dosing and to reduce temperatures in HTT processing of BSG. Mechanical dewatering of biomass can be an efficient method to increase solids loadings.

2.6.2.3 Efficient mechanical screw press dewatering

The techno-economic studies reported on xylitol production from BSG applied feedstock BSG material at 55% moisture content (González-García et al., 2018; Mussatto et al., 2013) yet BSG is disposed from breweries with significantly higher moisture contents (ca 75-85%) since the cost of further dewatering or steam drying is uneconomical (Huige, 1994). Therefore a dewatering step needs to be included in a techno-economic study of the valorisation concept of BSG. A mechanical screw press dewatering process step can be advantageous by simultaneous dewatering and fractionation through the removal of a fraction high in BSG protein (Schwencke, 2006). The incorporation of the protein product needs to be assessed as a possible by-production that can mitigate the cost of dewatering and improve the overall economics of the BSG valorisation concept of XOS and xylitol production.

Combining these possible improvements to reported studies, these will enable process simulations and techno-assessments that could improve the valorisation concept of BSG for the production of xylitol and XOS.

2.7 References

Aachary, A.A., Prapulla, S.G., 2011. Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Compr. Rev. Food Sci. Food Saf. 10, 2–16. https://doi.org/10.1111/j.1541-4337.2010.00135.x

Aboltins, A., Palabinskis, J., 2015. Research in Brewer's spent grain drying process. Eng. Rural Dev. 14, 230–235.

Abu-Ghannam, N., Balboa, E., 2018. 9 – Biotechnological, food, and health care applications, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier. https://doi.org/10.1016/B978-0-08-102162-0.00009-5

Acacio, K., Apyan, A., Araiza, R.M., Chiem, E., Kapaldo, J., Kim, P., Orekoya, M., Prusak, M., Sahni, S., Smith, A., Tomlin, S., Zahir, S., Dushay, M., Lewis, P., Marshall, T., Bottom, R., 2011. IPRO 340: Business Study of Alternative Uses for Brewers' Spent Grain.

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: Fundamentals toward application. Biotechnol. Adv. 29, 675–685. https://doi.org/10.1016/j.biotechadv.2011.05.005

Aguedo, M., Ruiz, H.A., Richel, A., 2015. Non-alkaline solubilization of arabinoxylans from destarched wheat bran using hydrothermal microwave processing and comparison with the hydrolysis by an endoxylanase. Chem. Eng. Process. Process Intensif. 96, 72–82. https://doi.org/10.1016/j.cep.2015.07.020

Allen, S.G., Schulman, D., Lichwa, J., Antal, M.J., Jennings, E., Elander, R., 2001. A Comparison of Aqueous and Dilute-Acid Single-Temperature Pretreatment of Yellow Poplar Sawdust. Ind. Eng. Chem. Res. 2352–2361.

Amorim, C., Silvério, S.C., Rodrigues, L.R., 2019. One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. Food Chem. 270, 86–94. https://doi.org/10.1016/j.foodchem.2018.07.080

Baik, B.K., Ullrich, S.E., 2008. Barley for food: Characteristics, improvement, and renewed interest. J. Cereal Sci. 48, 233–242. https://doi.org/10.1016/j.jcs.2008.02.002

Bajpai, P., 2016. Structure of Lignocellulosic Biomass, in: Pretreatment of Lignocellulosic Biomass for Biofuel Production. SpringerBriefs, pp. 7–12. https://doi.org/10.1007/978-981-10-0687-6

Bamforth, C.W., 2000. Review Brewing and brewing research : past , present and future. J. Sci. Food Agric. 80, 1371–1378.

Barakat, A., Mayer, C., Solhy, A., Arancon, R.A., De Vries, H., Luque, R., 2014. Mechanical Pretreatments of Lignocellulosic Biomass: towards facile and environmentally sound technologies for biofuels production. RSC Adv. 4, 48109–48127. https://doi.org/10.1039/C4RA07568D

Bartolomé, B., Santos, M., Jiménez, J.J., del Nozal, M.J., Gómez-Cordovés, C., 2002. Pentoses and hydroxycinnamic acids in brewer's spent grain. J. Cereal Sci. 36, 51–58. https://doi.org/10.1006/jcrs.2002.0442

Beldman, G., Hennekam, J., Voragen, A.G.J., 1987. Enzymatic Hydrolysis of Beer Brewers' Spent Grain and the Influence of Pretreatments. Biotechnol. Bioeng. 30, 668–671. https://doi.org/10.1017/S0021911800082024

Ben-Hamed, U., Seddighi, H., Thomas, K., 2011. Economic returns of using Brewery's spent grain in animal feed. World Acad. Sci. Eng. Technol. 50, 695–698.

Biddy, M.J., Scarlata, C., Kinchin, C., 2016. Chemicals from Biomass: A Market Assessment of Bioproducts with Near-Term Potential NREL/TP-5100-65509.

Borel, L.D.M.S., Lira, T.S., Ribeiro, J.A., Ataíde, C.H., Barrozo, M.A.S., 2018. Pyrolysis of brewer's spent grain: Kinetic study and products identification. Ind. Crops Prod. 121, 388–395. https://doi.org/10.1016/j.indcrop.2018.05.051

Borén, E., Larsson, S.H., Thyrel, M., Averheim, A., Broström, M., 2018. VOC off-gassing from pelletized steam exploded softwood bark: Emissions at different industrial process steps. Fuel Process. Technol. 171, 70–77. https://doi.org/10.1016/j.fuproc.2017.11.007

Brennan, A.H., Hoagland, W., Schell, D., Scott, C.D., 1986. High temperature acid hydrolysis of biomass using an engineering-scale plug flow reactor. Results of low testing solids, in: Biotechnol Bioeng Symp.

Briggs, D.E., Boulton, C., Brookes, P., Stevens, R., 2004. Brewing: Science and Practice. Woodhead Publishing Ltd.

Brink, M., 2004. Market and Product Assessment of Prebiotics and Probiotics strains for commertial use. University of Pretoria.

Brownell, H.H., Saddler, J.N., 1987. Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. Biotechnol. Bioeng. 29, 228–235. https://doi.org/10.1002/bit.260290213

Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam explosion pretreatment of wood: Effect of Chip Size, Acid, Moisture Content and Pressure Drop. Biotechnol. Bioeng. 28, 792–801. https://doi.org/10.1002/bit.260280604

Buffington, J., 2014. The Economic Potential of Brewer's Spent Grain (BSG) as a Biomass Feedstock. Adv. Chem. Eng. Sci. 308–318.

Cahela, D.R., Lee, Y.Y., Chambers, R.P., 1983. Modeling of percolation process in hemicellulose hydrolysis. Biotechnol. Bioeng. 25, 3–17. https://doi.org/10.1002/bit.260250103

Cardenas-toro, F.P., Alcazar-alay, S.C., Forster-carneiro, T., Meireles, M.A. a, 2014. Obtaining Oligo- and Monosaccharides from Agroindustrial and Agricultural Residues Using Hydrothermal Treatments. Food Public Heal. 4, 123–139. https://doi.org/10.5923/j.fph.20140403.08

Carrasco, C., Baudel, H.M., Sendelius, J., Modig, T., Roslander, C., Galbe, M., Hahn-Hägerdal, B., Zacchi, G., Lidén, G., 2010. SO2-catalyzed steam pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse. Enzyme Microb. Technol. 46, 64–73. https://doi.org/10.1016/j.enzmictec.2009.10.016

Carvalheiro, F., Duarte, L.C., Lopes, S., Parajo, J.C., Pereira, H., Girio, F.M., 2005a. Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by Debaryomyces hansenii CCMI 941. Process Biochem. 40, 1215–1223. https://doi.org/10.1016/j.procbio.2004.04.015

Carvalheiro, F., Duarte, L.C., Medeiros, R., Gírio, F.M., 2004a. Optimization of Brewery's Spent Grain Dilute-Acid Hydrolysis for the Production of Pentose-Rich Culture Media. Appl. Biochem. Biotechnol. 113–116, 1059–1072. https://doi.org/10.1385/ABAB:115:1-3:1059

Carvalheiro, F., Esteves, M.P., Parajo, J.C., Pereira, H., Gıırio, F.M., 2004b. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91, 93–100. https://doi.org/10.1016/S0960-8524(03)00148-2

Carvalheiro, F., Garrote, G., Parajo, J.C., Pereira, H., Gı, F.M., 2005b. Kinetic Modeling of Brewery's Spent Grain Autohydrolysis 233–243.

Carvalheiro, F., Garrote, G., Parajo, J.C., Pereira, H., Gı, F.M., 2005c. Kinetic Modeling of Brewery' s Spent Grain Autohydrolysis. Biotechnol.Prog. 21, 233–243.

Carvalho, A.F.A., Marcondes, W.F., de Oliva Neto, P., Pastore, G.M., Saddler, J.N., Arantes, V., 2018. The potential of tailoring the conditions of steam explosion to produce xylo-oligosaccharides from sugarcane bagasse. Bioresour. Technol. 250, 221–229. https://doi.org/10.1016/j.biortech.2017.11.041

Carvalho, A.F.A., Neto, P. de O., da Silva, D.F., Pastore, G.M., 2013. Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. Food Res. Int. 51, 75–85. https://doi.org/10.1016/j.foodres.2012.11.021

Celus, I., 2008. Characterisation and functionality of brewers' spent grain proteins and their enzymatic hydrolysates. Status Publ. Katholieke Universiteit Leuven.

Chemin, M., Wirotius, A.L., Ham-Pichavant, F., Chollet, G., Da Silva Perez, D., Petit-Conil, M., Cramail, H., Grelier, S., 2015. Well-defined oligosaccharides by mild acidic hydrolysis of hemicelluloses. Eur. Polym. J. 66, 190–197. https://doi.org/10.1016/j.eurpolymj.2015.02.008

Chen, H., 2015. Gas Explosion Technology and Biomass Refinery. Springer Netherlands.

Chen, J., Zhang, W., Zhang, H., Zhang, Q., Huang, H., 2014. Screw extrude steam explosion: A promising pretreatment of corn stover to enhance enzymatic hydrolysis. Bioresour. Technol. 161, 230–235. https://doi.org/10.1016/j.biortech.2014.02.043

Chen, R., Wu, Z., Lee, Y.Y., 1998. Shrinking-Bed Model for Percolation Process Applied to Dilute-Acid Pretreatment/Hydrolysis of Cellulosic Biomass. Appl. Biochem. Biotechnol. 70–72, 37–49.

Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst effects and the combined severity parameter. Appl. Biochem. Biotechnol. 24–25, 1–14. https://doi.org/10.1007/BF02920229

Church, J.A., Wooldridge, D., 1981. Continuous High-Solids Acid Hydrolysis of Biomass in a 11/2-in. Plug Flow Reactor. Ind. Eng. Chem. Prod. Res. Dev. 20, 371–378. https://doi.org/10.1021/i300002a027

Clauser, N.M., Gutiérrez, S., Area, M.C., Felissia, F.E., Vallejos, M.E., 2015. Small-sized biorefineries as strategy to add value to sugarcane bagasse. Chem. Eng. Res. Des. 107, 137–146. https://doi.org/10.1016/j.cherd.2015.10.050

Coelho, E., Rocha, M.A.M., Moreira, A.S.P., Domingues, M.R.M., Coimbra, M.A., 2016. Revisiting the structural features of arabinoxylans from brewers' spent grain. Carbohydr. Polym. 139, 167–176. https://doi.org/10.1016/j.carbpol.2015.12.006

Coelho, E., Rocha, M.A.M., Saraiva, J.A., Coimbra, M.A., 2014. Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides. Carbohydr. Polym. 99, 415–422. https://doi.org/10.1016/j.carbpol.2013.09.003

Collins, S., Reid, G., 2016. Distant site effects of ingested prebiotics. Nutrients 8, 1–20. https://doi.org/10.3390/nu8090523

Cook, D., 2011. Brewers' Grains: Opportunities abound. Brew. Guard. - Advant. Publ. Ltd, 60–63.

Cuevas, M., Saleh, M., García-Martín, J.F., Sáncheza, S., 2015. Influence of solid loading on D-xylose production through dilute sulphuric acid hydrolysis of olive stones. Int. J. Fats Oils 66, 635–646.

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of Initial Moisture Content and Chip Size on the Bioconversion Efficiency of Softwood Lignocellulosics. Biotechnol. Bioeng. 85, 413–421. https://doi.org/10.1002/bit.10905

Dávila, J.A., Rosenberg, M., Cardona, C.A., 2016. A biorefinery approach for the production of xylitol, ethanol and polyhydroxybutyrate from brewer's spent grain. AIMS Agric. Food 1, 52–66. https://doi.org/10.3934/agrfood.2016.1.52

De Albuquerque, T.L., Da Silva, I.J., De MacEdo, G.R., Rocha, M.V.P., 2014. Biotechnological production of xylitol from lignocellulosic wastes: A review. Process Biochem. 49, 1779–1789. https://doi.org/10.1016/j.procbio.2014.07.010

Diedericks, D., 2013. Extraction and Recovery of Precursor Chemicals From Sugarcane Bagasse , Bamboo and Triticale Bran Using Conventional , Advanced and Fractionation Pretreatment. Stellenbosch University.

Duarte, L.C., Carvalheiro, F., Lopes, S., Marques, S., Pajaró, J.C., Gírio, F.., 2004. Comparison of Two Posthydrolysis Processes of Brewery's Spent Grain Autohydrolysis Liquor to Produce a Pentose-Containing Culture Medium †. Appl. Biochem. Biotechnol. 113–116, 1041–1058.

E4Tech, 2015. From the Sugar Platform to biofuels and biochemicals Final report for the European Commission Directorate-General Energy N° ENER/C2/423-2012/SI2.673791.

Eggeman, T., Elander, R.T., 2005. Process and Economic Analysis of Pretreatment Technologies 96, 2019–25. https://doi.org/10.1017/CBO9781107415324.004

El-Shafey, E.I., Gameiro, M.L.F., Correia, P.F.M., de Carvalho, J.M.R., 2004. Dewatering of brewer's spent grain using a membrane filter press: A pilot plant study. Sep. Sci. Technol. 39, 3237–3261. https://doi.org/10.1081/SS-200028775

EPA, 1981. Source Category Survey: Animal Feed Dryers US EPA Report EPA-450/3-81-017.

Esslinger, H.M., 2009. Handbook of Brewing, Wiley-VCH Verlag GmbH. https://doi.org/10.1017/CBO9781107415324.004

Ewanick, S., Bura, R., 2011. The effect of biomass moisture content on bioethanol yields from steam pretreated switchgrass and sugarcane bagasse. Bioresour. Technol. 102, 2651–2658. https://doi.org/10.1016/j.biortech.2010.10.117

Fallourd, M.J., Viscione, L., 2009. Ingredient selection for stabilisation and texture optimisation of functional beverages and the inclusion of dietary fibre, in: Functional and Speciality Beverage Technology. Woodhead Publishers, pp. 3–38. https://doi.org/10.1533/9781845695569.1.3

Fan, X., Li, M., Zhang, J., Tang, P., Yuan, Q., 2014. Optimization of SO2-catalyzed hydrolysis of corncob for xylose and xylitol production. J. Chem. Technol. Biotechnol. 89, 1720–1726. https://doi.org/10.1002/jctb.4250

Fărcaş, A., Socaci, S., Tofană, M., Muresan, C., Mudara, E., Salanta, L., 2014. Nutritional properties and volitile profile brewer's spent grain supplemented bread. Rom. Biotechnol. Lett. 19, 9705–9714.

Fărcaş, A.C., 2014. Research Regarding the Identification and Exploitation of Biologically Active Compounds From Brewers' Spent GRain By-Product. University of Agricultural Sciences and Veterinary Medicine CLUJ-NAPOCA.

Fernandes Diniz, J.M.B., Gil, M.H., Castro, J.A.A.M., 2004. Hornification - Its origin and interpretation in wood pulps. Wood Sci. Technol. 37, 489–494. https://doi.org/10.1007/s00226-003-0216-2

Finley, J.W., Walker, C.E., Hautala, E., 1976. Utilization of Press Water from Brewers Spent Grains. J. Sci. Food Agric. 27, 655–660.

Franceschin, G., Sudiro, M., Ingram, T., Smirnova, I., Brunner, G., Bertucco, A., 2011. Conversion of rye straw into fuel and xylitol: A technical and economical assessment based on experimental data. Chem. Eng. Res. Des. 89, 631–640. https://doi.org/10.1016/j.cherd.2010.11.001

Fumi, M.D., Galli, R., Lambri, M., Donadini, G., Faveri, D.M. De, 2011. Effect of full-scale brewing process on polyphenols in Italian all-malt and maize adjunct lager beers. J. Food Compos. Anal. 24, 568–573. https://doi.org/10.1016/j.jfca.2010.12.006

Galbe, M., Zacchi, G., 2012. Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy 46, 70–78. https://doi.org/10.1016/j.biombioe.2012.03.026

Gallina, G., Alfageme, E.R., Biasi, P., García-Serna, J., 2018. Hydrothermal extraction of hemicellulose: from lab to pilot scale. Bioresour. Technol. 247, 980–991. https://doi.org/10.1016/j.biortech.2017.09.155

García-Aparicio, M., Parawira, W., Van Rensburg, E., Diedericks, D., Galbe, M., Rosslander, C., Zacchi, G., Görgens, J., 2011. Evaluation of steam-treated giant bamboo for production of fermentable sugars. Biotechnol. Prog. 27, 641–649. https://doi.org/10.1002/btpr.580

Garrido, R., 2012. The largest brewery in the world at Golden, Colorado [WWW Document]. Loyal. Travel. URL https://loyaltytraveler.boardingarea.com/2012/06/18/the-largest-brewery-in-the-world-at-golden-colorado/ (accessed 9.9.18).

Garrote, G., Domínguez, H., Parajó, J.C., 2002. Autohydrolysis of corncob: Study of non-isothermal operation for xylooligosaccharide production. J. Food Eng. 52, 211–218. https://doi.org/10.1016/S0260-8774(01)00108-X

Garrote, G., Domı, H., Parajo, J.C., 2004. Production of Substituted Oligosaccharides by Hydrolytic Processing of Barley Husks. Ind. Eng. Chem. Res. 43, 1608–1614.

GMI, 2017. Prebiotics Market worth over $8.5 Bn by 2024.

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

González-García, S., Morales, P.C., Gullón, B., 2018. Estimating the environmental impacts of a brewery waste–based biorefinery: Bio-ethanol and xylooligosaccharides joint production case study. Ind. Crops Prod. 123, 331–340. https://doi.org/10.1016/j.indcrop.2018.07.003

Guerrero, B.G., 2009. Effects of Amino Acid Profile, Endoprotease Activities and Wort Quality on Fermentability under Different Malting and Brewing Conditions. The University of Manitoba.

Gullón, P., González-Muñoz, M.J., Parajó, J.C., 2011. Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. Bioresour. Technol. 102, 6112–6119. https://doi.org/10.1016/j.biortech.2011.02.059

Gunes, B., Stokes, J., Davis, P., Connolly, C., Lawler, J., 2019. Pre-treatments to enhance biogas yield and quality from anaerobic digestion of whiskey distillery and brewery wastes: A review. Renew. Sustain. Energy Rev. 113, 109281. https://doi.org/10.1016/j.rser.2019.109281

Gurgel, L.V.A., Marabezi, K., Zanbom, M.D., Curvelo, A.A.D.S., 2012. Dilute acid hydrolysis of sugar cane bagasse at high temperatures: A Kinetic study of cellulose saccharification and glucose decomposition. Part I: Sulfuric acid as the catalyst. Ind. Eng. Chem. Res. 51, 1173–1185. https://doi.org/10.1021/ie2025739

Gurgel, L.V.A., Pimenta, M.T.B., Curvelo, A.A. da S., 2014. Enhancing liquid hot water (LHW) pretreatment of sugarcane bagasse by high pressure carbon dioxide (HP-CO2). Ind. Crops Prod. 57, 141–149. https://doi.org/10.1016/j.indcrop.2014.03.034

Heikkinen, S., 2016. Biodegradable films from cereal arabinoxylans. University of Helsinki.

Heitz, M., Capek-Ménard, E., Koeberle, P.G., Gagné, J., Chornet, E., Overend, R.P., Taylor, J.D., Yu, E., 1991. Fractionation of Populus tremuloides at the pilot plant scale: Optimization of steam pretreatment conditions using the STAKE II technology. Bioresour. Technol. 35, 23–32. https://doi.org/10.1016/0960-8524(91)90078-X

Hettrich, K., Drechsler, U., Loth, F., Volkert, B., 2017. Preparation and characterization of water-soluble xylan ethers. Polymers (Basel). 9, 1–13. https://doi.org/10.3390/polym9040129

Hongbin, C., Johann, G., Macintosh, P., Maria, G., 2014. Saccharification of bamboo by dilute acid pretreatment and enzymatic hydrolysis for cellulosic ethanol production. South African J. Chem. Eng. 19, 46–52.

Huige, N.J., 1994. Handbook of Brewing: In Food Science and Technology, in: Hardwick, W.A. (Ed.), Marcel Dekker Inc. CRC Press, pp. 501–550). https://doi.org/10.1002/9780470290118

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., Dudgeon, D., 2011. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol, National Renewable Energy Laboratory.

Ibbett, R., Gaddipati, S., Davies, S., Hill, S., Tucker, G., 2011. The mechanisms of hydrothermal deconstruction of lignocellulose : New insights from thermal – analytical and complementary studies. Bioresour. Technol. 102, 9272–9278. https://doi.org/10.1016/j.biortech.2011.06.044

Ikram, S., Huang, L., Zhang, H., Wang, J., Yin, M., 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 00, 1–11. https://doi.org/10.1111/1750-3841.13794

Ishiwaki, B.N., Murayama, H., Awayama, H., Kanauchi, O., Sato, T., 2000. Development of High Value Uses of Spent Grain by Fractionation Technology Q uarterly. MBAA Tech. Q. 37, 261–265.

Isikgor, F.H., Becer, C.R., 2015. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym. Chem. 6, 4497–4559. https://doi.org/10.1039/c5py00263j

Jacobsen, S.E., Wyman, C.E., 2002. Xylose Monomer and Oligomer Yields for Uncatalyzed Hydrolysis of Sugarcane Bagasse Hemicellulose at Varying Solids Concentration. Ind. Eng. Chem. Res. 41, 1454–1461. https://doi.org/10.1021/ie001025+

Jacobsen, S.E., Wyman, C.E., 2000. Cellulose and Hemicellulose Hydrolysis Models for Application to Current and Novel Pretreatment Processes. Appl. Biochem. Biotechnol. 84–86, 81–96. https://doi.org/10.1385/ABAB:84-86:1-9:81

Jacquet, N., Maniet, G., Vanderghem, C., Delvigne, F., Richel, A., 2015. Application of Steam Explosion as Pretreatment on Lignocellulosic Material: A Review. Ind. Eng. Chem. Res. 54, 2593–2598. https://doi.org/10.1021/ie503151g

Jay, A.J., Parker, M.L., Faulks, R., Husband, F., Wilde, P., Smith, A.C., Faulds, C.B., Waldron, K.W., 2008. A systematic micro-dissection of brewers' spent grain. J. Cereal Sci. 47, 357–364. https://doi.org/10.1016/j.jcs.2007.05.006

Jensen, J.R., Morinelly, J.E., Gossen, K.R., Brodeur-Campbell, M.J., Shonnard, D.R., 2010. Effects of dilute acid pretreatment conditions on enzymatic hydrolysis monomer and oligomer sugar yields for aspen, balsam, and switchgrass. Bioresour. Technol. 101, 2317–2325. https://doi.org/10.1016/j.biortech.2009.11.038

John, I., Yaragarla, P., Muthaiah, P., Ponnusamy, K., Appusamy, A., 2017. Statistical optimization of acid catalyzed steam pretreatment of citrus peel waste for bioethanol production. Resour. Technol. 3, 429–433. https://doi.org/10.1016/j.reffit.2017.04.001

Johnson, P., Paliwal, J., Cenkowski, S., 2010. Issues with utilisation of brewers' spent grain. Stewart Postharvest Rev. 6, 1–8. https://doi.org/10.2212/spr.2010.4.2

Kabel, M.A., Bos, G., Zeevalking, J., Voragen, A.G.J., Schols, H.A., 2007. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose

from wheat straw. Bioresour. Technol. 98, 2034–2042. https://doi.org/10.1016/j.biortech.2006.08.006

Kabel, M.A., Carvalheiro, F., Garrote, G., Avgerinos, E., Koukios, E., Parajó, J.C., Gírio, F.M., Schols, H.A., Voragen, A.G.J., 2002. Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. Carbohydr. Polym. 50, 47–56. https://doi.org/10.1016/S0144-8617(02)00045-0

Kapoor, M., Soam, S., Agrawal, R., Gupta, R.P., Tuli, D.K., Kumar, R., 2017. Pilot scale dilute acid pretreatment of rice straw and fermentable sugar recovery at high solid loadings. Bioresour. Technol. 224, 688–693. https://doi.org/10.1016/j.biortech.2016.11.032

Kemppainen, K., Rommi, K., Holopainen, U., Kruus, K., 2016. Steam explosion of Brewer's spent grain improves enzymatic digestibility of carbohydrates and affects solubility and stability of proteins. Appl. Biochem. Biotechnol. 1–15. https://doi.org/10.1007/s12010-016-2085-9

Kerby, C., Vriesekoop, F., 2017. An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3, 1–12. https://doi.org/10.3390/beverages3020024

Kim, K.H., Tucker, M.P., Keller, F. a, Aden, A., Nguyen, Q. a, 2001. Continuous countercurrent extraction of hemicellulose from pretreated wood residues. Appl. Biochem. Biotechnol. 91–93, 253–267. https://doi.org/10.1385/ABAB:91-93:1-9:253

Kim, T.H., Ryu, H.J., Oh, K.K., 2016. Low acid hydrothermal fractionation of Giant Miscanthus for production of xylose-rich hydrolysate and furfural. Bioresour. Technol. 218, 367–372. https://doi.org/10.1016/j.biortech.2016.06.106

Kim, Y., Hendrickson, R., Mosier, N., Ladisch, M., 2009. Liquid Hot Water Pretreatment of Cellulosic Biomass. Methods Mol. Biol. 581, 93–102. https://doi.org/10.1007/978-1-60761-214-8_7

Kokta, B.V., Ahmed, A., 1998. Steam Explosion Pulping, in: Young, R.A. (Ed.), Environmentally Friendly Technologies for the Pulp and Paper Industry. John Wiley & Sons Inc., pp. 191–211.

Kontturi, E., 2015. Hemicellulose: structure, characterization, dissolution, modification CHEM-E2140.

Krøll, E.B., 2017. Brewer's Spent Grains as A New Food Resource: Exploring the gastronomic and entrepreneurial potential of BSG. UN I V ERS I T Y OF COP ENHAGEN.

Kruse, A., Dahmen, N., 2015. Water - A magic solvent for biomass conversion. J. Supercrit. Fluids 96, 36–45. https://doi.org/10.1016/j.supflu.2014.09.038

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P., 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind. Eng. Chem. Res. 48, 3713–3729. https://doi.org/10.1021/ie801542g

Kupiainen, L., Ahola, J., Tanskanen, J., 2014. Kinetics of Formic Acid-catalyzed Cellulose Hydrolysis. BioResources 9, 2645–2658. https://doi.org/10.15376/biores.9.2.2645-2658

Laine, C., Kemppainen, K., Kuutti, L., Varhimo, A., Asikainen, S., Grönroos, A., Määttänen, M., Buchert, J., Harlin, A., 2015. Extraction of xylan from wood pulp and brewer's spent grain. Ind. Crops Prod. 70, 231–237. https://doi.org/10.1016/j.indcrop.2015.03.009

Larsen, J., Østergaard Petersen, M., Thirup, L., Li, H.W., Iversen, F.K., 2008. The IBUS process - Lignocellulosic bioethanol close to a commercial reality. Chem. Eng. Technol. 31, 765–772. https://doi.org/10.1002/ceat.200800048

Laser, M., Schulman, D., Allen, S.G., Lichwa, J., Antal, M.J., Lynd, L.R., 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour. Technol. 81, 33–44. https://doi.org/10.1016/S0960-8524(01)00103-1

LeBlanc, T., 2014. Why the FDA's Spent Grain Policy Nearly Raised the Cost of Your Beer [WWW Document]. Mod. Farmer. URL https://modernfarmer.com/2014/07/great-spent-grain-debacle/ (accessed 9.10.18).

Lee, Y.Y., Iyer, P., Xiang, Q., Hayes, J., 1998. Kinetic and Modeling Investigation to Provide Design Guidelines for the NREL Dilute- Acid Process Aimed at Total Hydrolysis / Fractionation of Lignocellulosic Biomass July 1998 Kinetic and Modeling Investigation to Provide Design Guidelines for the NREL D. NREL Tech. Rep.

Leibbrandt, N.H., Knoetze, J.H., Görgens, J.F., 2011. Comparing biological and thermochemical processing of sugarcane bagasse: An energy balance perspective. Biomass and Bioenergy 35, 2117–2126. https://doi.org/10.1016/j.biombioe.2011.02.017

Levic, J., Sredanovic, S., Levic, L., 2006. New feeds from brewery by-products. Roum. Biotechnol. Lett. 11, 2611–2618.

Li, H.Q., Jiang, W., Jia, J.X., Xu, J., 2014. pH pre-corrected liquid hot water pretreatment on corn stover with high hemicellulose recovery and low inhibitors formation. Bioresour. Technol. 153, 292–299. https://doi.org/10.1016/j.biortech.2013.11.089

Liao, W., Liu, Y., Liu, C., Chen, S., 2004. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material - Dairy manure. Bioresour. Technol. 94, 33–41. https://doi.org/10.1016/j.biortech.2003.11.011

Liao, Y.C., Yen, F.Y., Hung, F., Su, C.H., Chen, W.H., 2016. Intermittent pressurized operation of steam explosion pretreatment system. J. Taiwan Inst. Chem. Eng. 67, 285–291. https://doi.org/10.1016/j.jtice.2016.07.031

Liguori, R., Soccol, C.R., de Souza Vandenberghe, L.P., Woiciechowski, A.L., Faraco, V., 2015. Second generation ethanol production from brewers' spent grain. Energies 8, 2575–2586. https://doi.org/10.3390/en8042575

Linde, M., Galbe, M., Zacchi, G., 2006. Steam pretreatment of acid-sprayed and acid-soaked barley straw for production of ethanol. Appl. Biochem. Biotechnol. 129–132, 546–562. https://doi.org/10.1385/ABAB:130:1:546

Lischeske, J.J., Crawford, N.C., Kuhn, E., Nagle, N.J., Schell, D.J., Tucker, M.P., McMillan, J.D., Wolfrum, E.J., 2016. Assessing pretreatment reactor scaling through empirical analysis. Biotechnol. Biofuels 9, 213. https://doi.org/10.1186/s13068-016-0620-0

Liu, C., Wyman, C.E., 2004. The Effect of Flow Rate of Very Dilute Sulfuric Acid on Xylan, Lignin, and Total Mass Removal from Corn Stover. Ind. Eng. Chem. Res. 43, 2781–2788. https://doi.org/10.1021/ie030754x

López-Linares, J.C., García-Cubero, M.T., Lucas, S., González-Benito, G., Coca, M., 2019. Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production. Chem. Eng. J. 368, 1045–1055. https://doi.org/10.1016/j.cej.2019.03.032

Lopez, B., Pontiggia, R.M., Fernandez, H., 2010. Process for Drying Brewer's spent grains Spent Grains WO 2010053493. 2010053493.

Luft, L., Confortin, T.C., Todero, I., Ugalde, G., Zabot, G.L., Mazutti, M.A., 2018. Transformation of residual starch from brewer's spent grain into fermentable sugars using supercritical technology. J. Supercrit. Fluids 140, 85–90. https://doi.org/10.1016/j.supflu.2018.06.006

Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. J. Inst. Brew. 4, 553–568. https://doi.org/10.1002/jib.363

Machado, R.M., Rodrigues, R.A.D., Henriques, C.M.C., Gameiro, M.L.F., Ismael, M.R.C., Reis, M.T.A., Freire, J.P.B., Carvalho, J.M.R., 2016. Dewatering of brewer's spent grain using

an integrated membrane filter press with vacuum drying capabilities. Sep. Sci. Technol. 51, 692–700. https://doi.org/10.1080/01496395.2015.1117102

Mahnken, C.L., 2010. Utilization of Wet Brewers Grains As a Replacement for Corn Silage. KANSAS STATE UNIVERSITY.

Maiti, S., Gallastegui, G., Suresh, G., Laxman, V., Kaur, S., Le, Y., Drogui, P., Buelna, G., Verma, M., Galvez-cloutier, R., 2018. Microwave-assisted one-pot conversion of agro-industrial wastes into levulinic acid: An alternate approach. Bioresour. Technol. 265, 471–479. https://doi.org/10.1016/j.biortech.2018.06.012

Marston, S.P., 2007. Microbial and nutritional changes of wet brewers grains inoculated with a commercial preservative. University of New Hampshire.

Martinez-Hernandez, E., Tibessart, A., Campbell, G.M., 2018. Conceptual design of integrated production of arabinoxylan products using bioethanol pinch analysis. Food Bioprod. Process. 112, 1–8. https://doi.org/10.1016/j.fbp.2018.08.005

Martínez-Patiño, J.C., Romero-García, J.M., Ruiz, E., Oliva, J.M., Álvarez, C., Romero, I., Negro, M.J., Castro, E., 2015. High solids loading pretreatment of olive tree pruning with dilute phosphoric acid for bioethanol production by Escherichia coli. Energy and Fuels 29, 1735–1742. https://doi.org/10.1021/ef502541r

Mata, T.M., Tavares, T.F., Meireles, S., Caetano, N.S., 2015. Bioethanol from Brewers' Spent Grain: Pentose Fermentation. Chem. Eng. Trans. 43. https://doi.org/10.3303/CET1543041

Mathias, T.R.S., de Mello, P.P.M., Eliana, F.C.S., 2014. Solid wastes in brewing process: A review. J. Brew. Distill. 5, 1–9. https://doi.org/10.5897/JBD2014.0043

Matsumura, Y., 2015. Hydrothermal Gasification of Biomass, in: Recent Advances in Thermo-Chemical Conversion of Biomass. pp. 251–267.

Meneses, N.G.T., Martins, S., Teixeira, J.A., Mussatto, S.I., 2013. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. Sep. Purif. Technol. 108, 152–158. https://doi.org/10.1016/j.seppur.2013.02.015

Menukhov, N.V., 2006. Commodity evalation of chopped meat semis using spent grains. Disk and. Tech Sci. Kemerova 2005–2006.

Michelin, M., Teixeira, J.A., 2016. Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof. Bioresour. Technol. 216, 862–869. https://doi.org/10.1016/j.biortech.2016.06.018

Mitchell, H., 2006. Sweeteners and Sugar Alternatives in Food Technology. Blackwell Publishing.

Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment-A review. Biotechnol. Bioeng. 109, 1430–1442. https://doi.org/10.1002/bit.24464

Monin, T., 2016. Life Refreshment - Pilot for Environmentally Friendly, Efficient, Sustainable and Healthy products development: LIFE15 ENV/BE/000267.

Monschein, M., Nidetzky, B., 2016. Effect of pretreatment severity in continuous steam explosion on enzymatic conversion of wheat straw: Evidence from kinetic analysis of hydrolysis time courses. Bioresour. Technol. 200, 287–296. https://doi.org/10.1016/j.biortech.2015.10.020

Morinelly, J.E., Jensen, J.R., Browne, M., Co, T.B., Shonnard, D.R., 2009. Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass. Ind. Eng. Chem. Res. 48, 9877–9884. https://doi.org/10.1021/ie900793p

Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreshel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl. Biochem. Biotechnol. 125, 77–97. https://doi.org/10.1385/ABAB:125:2:077

Mosier, N.S., Ladisch, C.M., Ladisch, M.R., 2002. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. 79, 610–618. https://doi.org/10.1002/bit.10316

Moura, P., Carvalheiro, F., Esteves, M.P., Gírio, F.M., 2008. Prebiotic xylo-oligosaccharides as high-value co-products on an integrated biorefinery approach from lignocellulosic feedstock, in: International Conference and Exhibition on Bioenergy April 6th – 9th 2008 Universidade Do Minho, Guimarães, Portugal.

Mussatto, S.I., 2014. Brewer's spent grain: A valuable feedstock for industrial applications. J. Sci. Food Agric. 94, 1264–1275. https://doi.org/10.1002/jsfa.6486

Mussatto, S.I., Dragone, G., Roberto, I.C., 2006. Brewers' spent grain: Generation, characteristics and potential applications. J. Cereal Sci. 43, 1–14. https://doi.org/10.1016/j.jcs.2005.06.001

Mussatto, S.I., Moncada, J., Roberto, I.C., Cardona, C.A., 2013. Techno-economic analysis for brewer's spent grains use on a biorefinery concept: The Brazilian case. Bioresour. Technol. 148, 302–310. https://doi.org/10.1016/j.biortech.2013.08.046

Mussatto, S.I., Roberto, I.C., 2006. Chemical characterization and liberation of pentose sugars from brewer's spent grain. J. Chem. Technol. Biotechnol. 81, 268–274. https://doi.org/10.1002/jctb.1374

Mussatto, S.I., Roberto, I.C., 2005. Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J. Sci. Food Agric. 85, 2453–2460. https://doi.org/10.1002/jsfa.2276

Mussatto, S.I., Teixeira, J.A., 2010. Lignocellulose as raw material in fermentation processes. Appl. Microbiol. an Microb. Biotechnol. 2, 897–907. https://doi.org/10.1016/j.jrras.2014.02.003

Naidu, D.S., Hlangothi, S.P., John, M.J., 2018. Bio-based products from xylan: A review. Carbohydr. Polym. 179, 28–41. https://doi.org/10.1016/j.carbpol.2017.09.064

Nieder-Heitmann, M., Haigh, K.F., Görgens, J.F., 2018. Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses. Bioresour. Technol. 262, 159–168. https://doi.org/10.1016/j.biortech.2018.04.075

Niemi, P., Tamminen, T., Smeds, A., Viljanen, K., Ohra-Aho, T., Holopainen-Mantila, U., Faulds, C.B., Poutanen, K., Buchert, J., 2012. Characterization of lipids and lignans in brewer's spent grain and its enzymatically extracted fraction. J. Agric. Food Chem. 60, 9910–9917. https://doi.org/10.1021/jf302684x

NutraSource, I., 2013. GRAS Notification (GRN) No 458 for Shangdong Longlive Biotechnology, Ltd.'s Xylooligosaccharides.

Öhgren, K., Galbe, M., Zacchi, G., 2005. Optimization of Steam Pretreatment of SO 2 - Impregnated Corn Stover for Fuel Ethanol Production. Appl. Biochem. Biotechnol. 121.

Oliver, G., 2011. The Oxford Companion to Beer. Oxford University Press.

Olsson, L., 2007. Biofuels, Advances in Biochemical engineering/Biotechnology. https://doi.org/10.1016/j.jbiotec.2007.09.006

Ouyang, X., Wang, W., Yuan, Q., Li, S., Zhang, Q., Zhao, P., 2015. Improvement of lignin yield and purity from corncob in the presence of steam explosion and liquid hot pressured alcohol. RSC Adv. 5, 61650–61656. https://doi.org/10.1039/C5RA12452B

Overend, R., Chornet, E., Gascoigne, J.A., 1987. Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments [and Discussion], Philosophical Transactions of The Royal Society A: Mathematical, Physical and Engineering Sciences. https://doi.org/10.1098/rsta.1987.0029

Özüdoğru, H.M.R., 2018. Techno-economic Analysis of Xylitol, Citric Acid and Glutamic Acid Biorefinery Scenarios Utilizing Sugarcane Lignocellulose. Stellenbosch University.

Palmer, G.H., 1992. Cereal science and malting technology: the future. J. Am. Soc. Brew. Chem. 50, 121–130. https://doi.org/10.1094/ASBCJ-50-0121

Pauli, G., 2015. Clustering Beer, Energy and Food [WWW Document]. Another Case Blue Econ. - CASE 109. URL https://www.theblueeconomy.org/uploads/7/1/4/9/71490689/case_109_clustering_beer_energy_and_food_final_4_2015.pdf

Pedersen, M., Johansen, K.S., Meyer, A.S., 2011. Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw. Biotechnol. Biofuels 4, 11. https://doi.org/10.1186/1754-6834-4-11

Pedersen, M., Meyer, A.S., 2010. Lignocellulose pretreatment severity - relating pH to biomatrix opening. N. Biotechnol. 27, 739–750. https://doi.org/10.1016/j.nbt.2010.05.003

Persson, T., Dinh, E., Jönsson, A.S., 2009. Improvement of arabinoxylan isolation from barley husks. Food Bioprod. Process. 87, 228–233. https://doi.org/10.1016/j.fbp.2008.10.001

Pierre, G., Sannier, F., Goude, R., Nouviaire, A., Maache-Rezzoug, Z., Rezzoug, S.A., Maugard, T., 2011. Evaluation of thermomechanical pretreatment for enzymatic hydrolysis of pure microcrystalline cellulose and cellulose from Brewers' spent grain. J. Cereal Sci. 54, 305–310. https://doi.org/10.1016/j.jcs.2011.06.004

Pinheiro, T., Coelho, E., Romaní, A., Domingues, L., 2019. Intensifying ethanol production from brewer's spent grain waste: Use of whole slurry at high solid loadings. N. Biotechnol. 53, 1–8. https://doi.org/10.1016/j.nbt.2019.06.005

Plaza, P.E., Gallego-Morales, L.J., Peñuela-Vásquez, M., Lucas, S., García-Cubero, M.T., Coca, M., 2017. Biobutanol production from brewer's spent grain hydrolysates by Clostridium beijerinckii. Bioresour. Technol. 244, 166–174. https://doi.org/10.1016/j.biortech.2017.07.139

Poelmans, E., Swinnen, J.F.M., 2011. From Monasteries to Multinationals (and Back): A Historical Review of the Beer Economy, LICOS Discussion Paper, No. 294, LICOS, Leuven.

Procentese, A., Raganati, F., Olivieri, G., Russo, M.E., Rehmann, L., Marzocchella, A., 2018. Deep Eutectic Solvents pretreatment of agro-industrial food waste. Biotechnol. Biofuels 11, 1–12. https://doi.org/10.1186/s13068-018-1034-y

Qihua, D., Li, F., Deliang, W., Xia, L., Zhiping, L., Fengchao, J., 2010. Research into Pretreatment Methods of Brewer's Spent Grain (Chinese). Food Ferment. Ind. 36, 116–121.

Qin, F., Johansen, A.Z., Mussatto, S.I., 2018. Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains. Ind. Crops Prod. 125, 443–453. https://doi.org/10.1016/j.indcrop.2018.09.017

Rafiqul, I.S.M., Sakinah, A.M.M., 2013. Processes for the Production of Xylitol—A Review. Food Rev. Int. 29, 127–156. https://doi.org/10.1080/87559129.2012.714434

Ramukhwatho, F., Seetal, A., Pienaar, H., 2016. NATSURV 1: Water and Wastewater Management in the Malt Brewing Industry (Ed 2) WRC Report No. TT 676/16. Pretoria.

Rao, L. V., Goli, J.K., Gentela, J., Koti, S., 2015. Bioconversion of lignocellulosic biomass to xylitol: An overview. Bioresour. Technol. 213, 299–310. https://doi.org/10.1016/j.biortech.2016.04.092

Rastall, R.A., Gibson, G.R., 2015. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Curr. Opin. Biotechnol. 32, 42–46. https://doi.org/10.1016/j.copbio.2014.11.002

Ravindran, R., Jaiswal, S., Abu-ghannam, N., Jaiswal, A.K., 2018. A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers ' spent grain. Bioresour. Technol. 248, 272–279. https://doi.org/10.1016/j.biortech.2017.06.039

Reis, S.F., Coelho, E., Coimbra, M.A., Abu-Ghannam, N., 2015. Influence of grain particle sizes on the structure of arabinoxylans from brewer's spent grain. Carbohydr. Polym. 130, 222–6. https://doi.org/10.1016/j.carbpol.2015.05.031

Reis, S.F., Gullón, B., Gullón, P., Ferreira, S., Maia, C.J., Alonso, J.L., Domingues, F.C., Abu-Ghannam, N., 2014. Evaluation of the prebiotic potential of arabinoxylans from brewer's spent grain. Appl. Microbiol. Biotechnol. 98, 9365–9373. https://doi.org/10.1007/s00253-014-6009-8

Ren, J., Wang, S., Gao, C., Chen, X., Li, W., Peng, F., 2015. TiO2-containing PVA/xylan composite films with enhanced mechanical properties, high hydrophobicity and UV shielding performance. Cellulose 22, 593–602. https://doi.org/10.1007/s10570-014-0482-1

Rencoret, J., Prinsen, P., Gutiierrez, A., Martinez, A.T., Del Rio, J.C., 2015. Isolation and structural characterization of the milled wood lignin, dioxane lignin, and cellulolytic lignin preparations from brewer's spent grain. J. Agric. Food Chem. 63, 603–613. https://doi.org/10.1021/jf505808c

REPRO, 2008. Reducing Food Processing Waste REPRO CT-2005-006922.

Ribeiro, L.S., Delgado, J.J., de Melo Órfão, J.J., Ribeiro Pereira, M.F., 2016. A one-pot method for the enhanced production of xylitol directly from hemicellulose (corncob xylan). RSC Adv. 6, 95320–95327. https://doi.org/10.1039/C6RA19666G

Richard, A., D'Agostino, D., Davies, C., Plooy, A. Du, Kerr, I., 1998. Steam explosion pulping of bagasse for fine paper production, in: Tappi Pulping Conference 1998. TAPPI PRESS, pp. 1053–1062.

Robertson, J.A., I'Anson, K.J.A., Brocklehurst, T.F., Faulds, C.B., Waldron, K.W., 2010a. Effect of storage conditions on the microbial ecology and biochemical stability of cell wall components in brewers spent grain. J. Agric. Food Chem. 58, 7266–7272. https://doi.org/10.1021/jf1001099

Robertson, J.A., I'Anson, K.J.A., Treimo, J., Faulds, C.B., Brocklehurst, T.F., Eijsink, V.G.H., Waldron, K.W., 2010b. Profiling brewers' spent grain for composition and microbial ecology at the site of production. LWT - Food Sci. Technol. 43, 890–896. https://doi.org/10.1016/j.lwt.2010.01.019

Rojas-Chamorro, J.A., Cara, C., Romero, I., Ruiz, E., Romero-García, J.M., Mussatto, S.I., Castro, E., 2018. Ethanol Production from Brewers' Spent Grain Pretreated by Dilute Phosphoric Acid. Energy & Fuels 32, 5226–5233. https://doi.org/10.1021/acs.energyfuels.8b00343

Rojas Pérez, L.C., 2018. Valorisation of brewers spent grains in the production of xylitol (Spanish). Universidad Nacional de Colombia.

Romdhana, H., Bonazzi, C., Esteban-Decloux, M., 2015. Superheated Steam Drying: An Overview of Pilot and Industrial Dryers with a Focus on Energy Efficiency. Dry. Technol. 33, 1255–1274. https://doi.org/10.1080/07373937.2015.1025139

Rommi, K., Niemi, P., Kemppainen, K., Kruus, K., 2018. Impact of thermochemical pre-treatment and carbohydrate and protein hydrolyzing enzyme treatment on fractionation of protein and lignin from brewer's spent grain. J. Cereal Sci. 79, 168–173. https://doi.org/10.1016/j.jcs.2017.10.005

Roos, A.A., Persson, T., Krawczyk, H., Zacchi, G., Stålbrand, H., 2009. Extraction of water-soluble hemicelluloses from barley husks. Bioresour. Technol. 100, 763–769. https://doi.org/10.1016/j.biortech.2008.07.022

Rosa, M., Beloborodko, A., 2015. A decision support method for development of industrial synergies : case studies of Latvian brewery and wood-processing industries. J. Clean. Prod. 105, 461–470. https://doi.org/10.1016/j.jclepro.2014.09.061

Sajib, M., Falck, P., Sardari, R.R.R., Mathew, S., Grey, C., Karlsson, E.N., Adlercreutz, P., 2018. Valorization of Brewer's spent grain to prebiotic oligosaccharide: production, xylanase catalyzed hydrolysis, in-vitro evaluation with probiotic strains and in a batch human fecal fermentation model. J. Biotechnol. 268, 61–70. https://doi.org/10.1016/j.jbiotec.2018.01.005

Sanni, L.A., Fakunle, O.A., 2016. Effect of Dryer Parameters on Drying Rate and Quality of Spent Brewers ' Grain. Int. J. Sci. Technoledge 4, 108–112.

Santos, M., Jiménez, J.J., Bartolomé, B., Gómez-Cordovés, C., Del Nozal, M.J., 2003. Variability of brewer's spent grain within a brewery. Food Chem. 80, 17–21. https://doi.org/10.1016/S0308-8146(02)00229-7

Scheller, L., Michel, R., 2012. Brewing Beer – efficient and sustainable technologies in regard to energy and raw material input, in: ERSCP Workshop, Bregenz, 3 May 2012. GEA Brewery Systems Huppmann Tuchenhagen, Bregenz, pp. 1–38.

Schneider, J., Krottenthaler, M., Back, W., Weisser, H., Brew, J.I., 2005. Study on the Membrane Filtration of Mash with Particular Respect to the Quality of Wort and Beer. J. Inst. Brew.

Schwencke, K. V, 2006. Sustainable, Cost-Effective, and Feasible Solutions for the Treatment of Brewers' Spent Grains. MBAA Tech. Q. 43, 199–202.

Severini, C., Azzollini, D., Jouppila, K., Jussi, L., Derossi, A., De Pilli, T., 2015. Effect of enzymatic and technological treatments on solubilisation of arabinoxylans from brewer's spent grain. J. Cereal Sci. 65, 162–166. https://doi.org/10.1016/j.jcs.2015.07.006

Shao, X., Lynd, L., 2013. Kinetic modeling of xylan hydrolysis in co- and countercurrent liquid hot water flow-through pretreatments. Bioresour. Technol. 130, 117–124. https://doi.org/10.1016/j.biortech.2012.11.109

Shen, J., Wyman, C.E., 2011. A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. Bioresour. Technol. 102, 9111–9120. https://doi.org/10.1016/j.biortech.2011.04.001

Shindo, S., Tachibana, T., 2006. Production of Bioethanol from Spent Grainn— A By-Product of Beer Production. J. Inst. Brew. 43, 189–193.

Shindo, S., Tachibana, T., 2004. Production of L-Lactic Acid from Spent Grain, a By-Product of Beer Production. J. Inst. Brew. 110, 347–351.

Skendi, A., Harasym, J., Galanakis, C.M., 2018. 7 – Recovery of high added-value compounds from brewing and distillate processing by-products, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier Science. https://doi.org/10.1016/B978-0-08-102162-0.00007-1

Steele, E.A., 2010. Wheat Bran Extract: GRAS Notice 000343.

Steiner, J., Franke, K., Kießling, M., Fischer, S., Töp, S., Heinz, V., Becker, T., 2018. Influence of hydrothermal treatment on the structural modification of spent grain specific carbohydrates and the formation of degradation products using model compounds. Carbohydr. Polym. 184, 315–322. https://doi.org/10.1016/j.carbpol.2017.12.038

Steiner, J., Procopio, S., Becker, T., 2015. Brewer's spent grain: source of value-added polysaccharides for the food industry in reference to the health claims. Eur. Food Res. Technol. 303–315. https://doi.org/10.1007/s00217-015-2461-7

Stelte, W., 2013. Steam explosion for biomass pre-treatment, Danish Technological Institute.

Stepan, A.M., 2013. Bioplastics from Biomass - Acetylation of Xylans with Green Chemistry. CHALMERS UNIVERSITY OF TECHNOLOGY.

Stiles, R.S., Herbert, H.S., 1977. Centrifugal Separation, Isolation and Characteristics of Brewery Spent Grains and Press Liquor. MBAA Tech. Q. 14, 21–34.

Stroem, L.K., Desai, D.K., Hoadley, A.F.A., 2009. Superheated steam drying of Brewer's spent grain in a rotary drum. Adv. Powder Technol. 20, 240–244. https://doi.org/10.1016/j.apt.2009.03.009

Ström, L.-L., n.d. Evaluation of Pilot Scale Drying of Brewer's Spent Grain in a Keith Rotary Superheated Steam Dryer -a Preliminary Investigation.

Sui, W., Chen, H., 2015. Study on loading coefficient in steam explosion process of corn stalk. Bioresour. Technol. 179, 534–542. https://doi.org/10.1016/j.biortech.2014.12.045

Tao, L., Aden, A., Elander, R.T., Pallapolu, V.R., Lee, Y.Y., Garlock, R.J., Balan, V., Dale, B.E., Kim, Y., Mosier, N.S., Ladisch, M.R., Falls, M., Holtzapple, M.T., Sierra, R., Shi, J., Ebrik, M.A., Redmond, T., Yang, B., Wyman, C.E., Hames, B., Thomas, S., Warner, R.E., 2011. Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass. Bioresour. Technol. 102, 11105–11114. https://doi.org/10.1016/j.biortech.2011.07.051

Teng, C., Yan, Q., Jiang, Z., Fan, G., Shi, B., 2010. Production of xylooligosaccharides from the steam explosion liquor of corncobs coupled with enzymatic hydrolysis using a thermostable xylanase. Bioresour. Technol. 101, 7679–7682. https://doi.org/10.1016/j.biortech.2010.05.004

Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., 1998. Comparison of SO2 and H2SO4 impregnation of softwood prior to steam

pretreatment on ethanol production. Appl. Biochem. Biotechnol. 70–72, 3–15. https://doi.org/10.1007/BF02920119

Thomas, K.R., Rahman, P.K.S.M., 2006. Brewery wastes. Strategies for sustainability. A review. Asp. Appl. Biol. 80, 147–153.

Thompson, D.R., Grethlein, H.E., 1979. Design and Evaluation of a Plug Flow Reactor for Acid Hydrolysis of Cellulose. Ind. Eng. Chem. Prod. Res. Dev. 18, 166–169. https://doi.org/10.1021/i360071a003

Torres-Mayanga, P.C., Azambuja, S.P.H., Tyufekchiev, M., Tompsett, G.A., Timko, M.T., Goldbeck, R., Rostagno, M.A., Forster-Carneiro, T., 2019. Subcritical water hydrolysis of brewer's spent grains: Selective production of hemicellulosic sugars (C-5 sugars). J. Supercrit. Fluids 145, 19–30. https://doi.org/10.1016/j.supflu.2018.11.019

Towler, G.P., Sinnott, R.K., 2008. Chemical Engineering Design: Principles, Practice and Economics of Plant and Process Design. Elsevier.

Ur-Rehman, S., Mushtaq, Z., Zahoor, T., Jamil, A., Anjum Murtaza, M., 2013. Xylitol; A review on Bio-production, Application, Health Benefits and Related Safety Issues. Crit. Rev. Food Sci. Nutr. 55, 37–41. https://doi.org/10.1080/10408398.2012.702288

Vallejos, M.E., Zambon, M.D., Area, M.C., Curvelo, A.A. da S., 2012. Low liquid–solid ratio (LSR) hot water pretreatment of sugarcane bagasse. Green Chem. 14, 1982. https://doi.org/10.1039/c2gc35397k

Vavpot, V.J., Williams, R.J., Williams, M.A., 2014. Green Vegetable Oil Processing, Green Vegetable Oil Processing. Elsevier. https://doi.org/10.1016/B978-0-9888565-3-0.50004-2

Vazquez, M.J., Alonso, J.L., Dominquez, H., Pajaro, J.C., 2000. Xylooligo-saccharides: manufacture and applications. Trends Food Sci. Technol. 11, 387–393.

Vegas, R., Alonso, L., Domıniques, H., Parajó, J.C., 2005. Manufacture and Refining of Oligosaccharides from Industrial Solid Wastes. Ind. Eng. Chem. Res 44, 614–620.

Verardi, A., Bari, I. De, Ricca, E., Calabrò, V., 2012. Hydrolysis of Lignocellulosic Biomass: Current Status of Processes and Technologies and Future Perspectives, in: Lima, M.A.P. (Ed.), Bioethanol. InTech, pp. 96–122. https://doi.org/10.5772/23987

Wang, B., Luo, Y., Myung, K.H., Liu, J.X., 2014. Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grains. Asian-Australasian J. Anim. Sci. 27, 832–840. https://doi.org/10.5713/ajas.2013.13668

Wang, C., 2014. Renewable Natural Polymer Thin Films and Their Interactions with Biomacromolecules. Virginia Polytechnic Institute and State University.

Wang, L., Fan, X., Tang, P., Yuan, Q., 2013. Xylitol fermentation using hemicellulose hydrolysate prepared by acid pre-impregnated steam explosion of corncob. J. Chem. Technol. Biotechnol. 88, 2067–2074. https://doi.org/10.1002/jctb.4070

Waters, D.M., Jacob, F., Titze, J., Arendt, E.K., Zannini, E., 2012. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. Eur. Food Res. Technol. 235, 767–778. https://doi.org/10.1007/s00217-012-1805-9

Weber, B., Stadlbauer, E.A., 2017. Sustainable paths for managing solid and liquid waste from distilleries and breweries. J. Clean. Prod. 149, 38–48. https://doi.org/10.1016/j.jclepro.2017.02.054

Weger, A., Binder, S., Franke, M., Hornung, A., 2014. Solid Biofuel Production by Mechanical Pre-Treatment of Brewers' Spent Grain. Chem. Eng. Trans. 37, 661–666. https://doi.org/10.3303/CET1437111

Weger, A., Jung, R., Stenzel, F., Hornung, A., 2017. Optimized Energetic Usage of Brewers' Spent Grains. Chem. Eng. Technol. 40, 306–312. https://doi.org/10.1002/ceat.201600186

Wei, H., Chen, X., Shekiro, J., Kuhn, E., Wang, W., Ji, Y., Kozliak, E., Himmel, M., Tucker, M., 2018. Kinetic Modelling and Experimental Studies for the Effects of Fe2+ Ions on Xylan Hydrolysis with Dilute-Acid Pretreatment and Subsequent Enzymatic Hydrolysis. Catalysts 8, 39. https://doi.org/10.3390/catal8010039

Weil, J.R., Sarikaya, A., Rau, S.-L., Goetz, J., Ladisch, C.M., Brewer, M., Hendrickson, R., Ladisch, M.R., 1998. Pretreatment of corn fiber by pressure cooking in water. Appl. Biochem. Biotechnol. 73, 1–17. https://doi.org/10.1007/BF02788829

White, J.S., Yohannan, B.K., Walker, G.M., 2008. Bioconversion of brewer's spent grains to bioethanol. FEMS Yeast Res. 8, 1175–1184. https://doi.org/10.1111/j.1567-1364.2008.00390.x

Wilhelmson, A., Lehtinen, P., VonWeymarn, N., Itävaara, M., Sibakov, J., Heiniö, R.-L., Forssell, P., Buchert, J., 2009. Future applications for brewers' spent grain. New Food.

Wilkinson, S., Smart, K. a., Cook, D.J., 2014. A Comparison of Dilute Acid- and Alkali-Catalyzed Hydrothermal Pretreatments for Bioethanol Production from BSG. J Am Soc Brew Chem 72, 143–153.

Wilkinson, S., Smart, K.A., Cook, D.J., 2015. Optimising the (Microwave) Hydrothermal Pretreatment of Brewers Spent Grains for Bioethanol Production. J. Fuels 2015. https://doi.org/http://dx.doi.org/10.1155/2015/369283

Wilkinson, S., Smart, K.A., James, S., Cook, D.J., 2016. Maximising high solid loading enzymatic saccharification yield from acid-catalysed hydrothermally-pretreated brewers spent grain. Biofuel Res. J. 3, 417–429. https://doi.org/10.18331/BRJ2016.3.2.7

Williams, L., 2013. Other Water Treatment Plant Residuals Dewatering Equipment: An Investigation of Screw Press Technology.

Wilson, J., 1973. Method of treating spent grain US 3721568 A. US 3721568 A.

Wu, H., Fu, Q., Giles, R., Bartle, J., 2008. Production of mallee biomass in Western Australia: Energy balance analysis. Energy and Fuels 22, 190–198. https://doi.org/10.1021/ef7002969

Yan, Q., Miazek, K., Grande, P.M., Domínguez de María, P., Leitner, W., Modigell, M., 2014. Mechanical pretreatment in a screw press affecting chemical pulping of lignocellulosic biomass. Energy & Fuels 28, 6981–6987. https://doi.org/10.1021/ef501706w

Yan, Q., Modigell, M., 2012. Mechanical pretreatment of lignocellulosic biomass using a screw press as an essential step in the biofuel production. Chem. Eng. Trans. 29, 601–606. https://doi.org/10.3303/CET1229101

Yang, B., Gray, M.C., Liu, C., Lloyd, T.A., Stuhler, S.L., Converse, A.O., Wyman, C., 2004. Unconventional Relationships for Hemicellulose Hydrolysis and Subsequent Cellulose Digestion, in: Lignocellulose Biodegradation. American Chemical Society.

Yang, B., Tucker, M., 2013. Laboratory Pretreatment Systems to Understand Biomass Deconstruction, in: E., C., Wyman (Eds.), Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals. John Wiley & Sons Inc., pp. 489–521. https://doi.org/10.1002/9780470975831.ch23

Yang, B., Wyman, C.E., 2009. Dilute Acid and Autohydrolysis Pretreatment, in: Mielenz, J.R. (Ed.), Biofuels: Methods and Protocols, Methods in Molecular Biology. Springer, pp. 103–114. https://doi.org/10.1007/978-1-60761-214-8

Zhang, H.J., Fan, X.G., Qiu, X.L., Zhang, Q.X., Wang, W.Y., Li, S.X., Deng, L.H., Koffas, M.A.G., Wei, D.S., Yuan, Q.P., 2014. A novel cleaning process for industrial production of xylose in pilot scale from corncob by using screw-steam-explosive extruder. Bioprocess Biosyst. Eng. 2425–2436. https://doi.org/10.1007/s00449-014-1219-0

3. Research scope

The scope of the project is to significantly improve on existing BSG HTT processing technology to support the valorisation concept of BSG through the production of high value products for food applications, primarily XOS and xylitol. Process intensification would be investigated through application of a screw press dewatering step to lower moisture content in BSG before using two HTT technologies for fractionation, namely (i) a bench scale high pressure stirred reactor (PARR), and (ii) pilot scale steam explosion reactor. The bench scale stirred reactor (i) would be suitable for BSG HTT optimisation investigations using acid catalyst dosing such as extremely low acid (ELA) dosing while operating at low solids loadings (<15% dm). The pilot scale steam explosion reactor (ii) would be suitable for BSG HTT scale up investigations suitable for high solids (>25% dm) HTT. Both HTT technologies investigated would generate novel insight through characterising relationships between process variables, ELA, BSG composition and screws press dewatering for XOS production including XOS yields, xylobiose and xylotriose fraction in the XOS, xylose sugar yields, total dissolved solids (TDS), xylan recovery and inhibitors. The overall aim is to use this data to improve the techno-economics of a concept process for BSG valorisation.

3.1 Aims and objectives

The aim of the project was to develop significantly improved valorisation concepts of BSG for a small scale biorefinery co-located at a brewery through the production of high-value products for food applications, specifically through XOS and/or xylitol, while taking into consideration BSG availability. This aim would be achieved by developing improved HTT processing technology used for XOS and xylose production from BSG, firstly, by conducting experimental research for BSG HTT process intensification through the use of increased (>11% dm) dry matter concentration achieved by mechanical dewatering BSG and the optimisation of extremely low acid (ELA) dosing. Secondly by the scale up of high solids loadings BSG HTT processing technology for fractionation of BSG hemicellulose for production of both XOS and xylose (for xylitol production). Lastly, the best valorisation concept of BSG from three process flow scenarios, for production of XOS, xylitol and both together was established by comparing the capital expenditure and profitability of each scenario by techno-economical assessments. Therefore, to achieve the overall aim, two main objectives were defined; firstly for experimental work on the HTT technology development and secondly the techno-economical assessments. The work flow is graphically represented in Figure 3-2.

3.1.1 Key objectives of the work

The objectives of the work are outlined as follows:

A. Objective #1: HTT techniques for fractionation of BSG in an energy efficient manner, by using high dry matter contents

Screw press dewatering of BSG for increased solids loading

1. Apply a screw press to efficiently increase the dry matter content of raw BSG (two types Weiss and Malt) to enable HTT investigations with high solids-loading. Moisture reductions from raw BSG by the screw press will be sought to two levels:
   (i) A *circa* 50% reduction in moisture from raw BSG and
   (ii) B another 50% reduction in moisture from level A.

Bench scale stirred batch reactor work

2. Benchmark HTT treatment conditions for XOS and xylose production based from literature (11% dm) for both raw and screw pressed BSG (Level A) in;
   (i) Autocatalytic (No added acid) HTT for preferred conditions of 180 ºC 12.2 min) and optimum conditions for maximum XOS production (Gomez *et al.*, 2015).
   (ii) Dilute acid HTT conditions (100 mg H_2SO_4/g 121 ºC 17 min) for maximum xylose for xylitol production (Mussatto and Roberto, 2005).

3. Considering (i) and (ii) and other conditions in between (Figure 3-1), select an appropriate range of process variables (residence time, temperature, acid loadings or ELA dosing) to optimise XOS production and minimisation of degradation products from raw BSG and screw pressed BSG (level A).

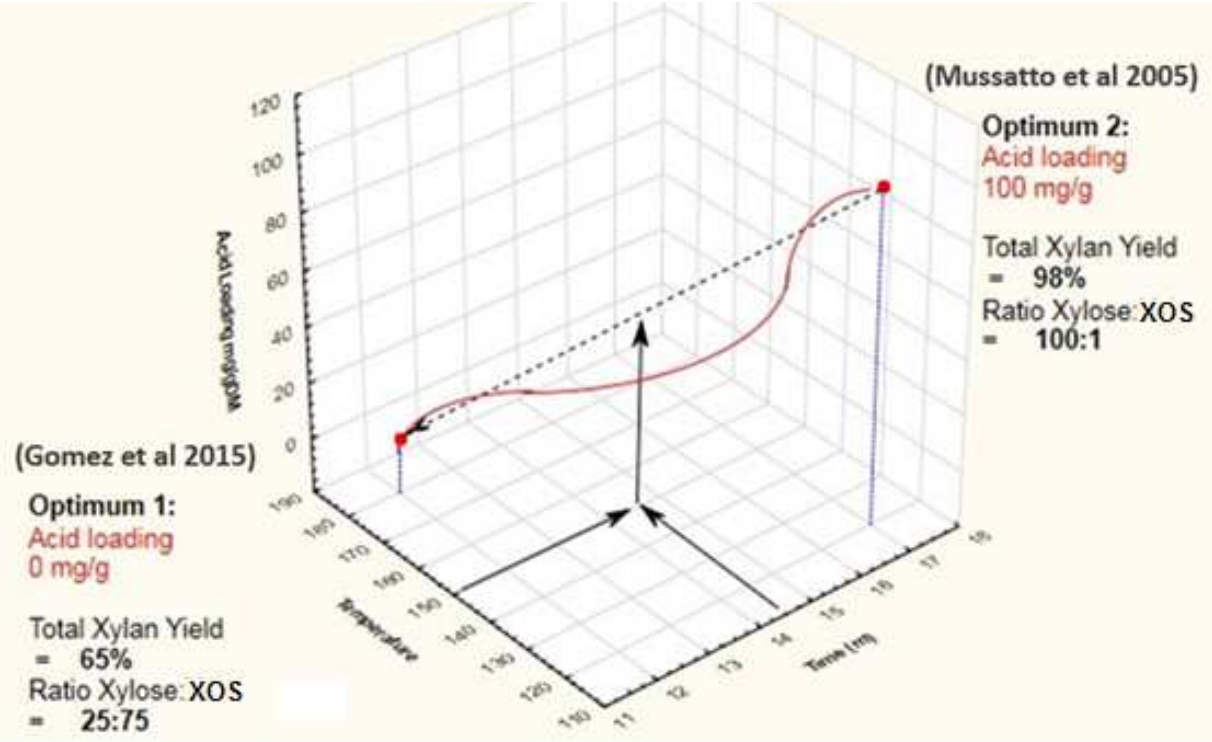


Figure 3-1 HTT process variable space: Time, Temperature and Acid loading

4. Conduct a factorial experimental design in the selected range for process variable (temperature, acid loadings or ELA dosing and residence time) for optimisation of XOS and xylose production for each, raw and screw pressed BSG (level A)
   (i) Establish relationships between input variables to predict XOS yields, xylose sugar yields, xylobiose, xylotriose, TDS, xylan recovery and degradation by-products or fermentation inhibitors from both raw and screw pressed BSG (level A)
   (ii) Determine if CO_2 catalyst addition at temperatures (150 ºC) and pressures (20 Bar) can be used to significantly increase HTT severity.

5. Determine the effect of the screw press dewatering on HTT by comparing the pH, CSF and acidification in HTT (mol H+ released per dry gram of BSG) between raw and screw pressed BSG (level A) with two HTT comparisons:
   (i) Autocatalytic (no acid added) HTT (at 180 ºC and 150 ºC) runs and
   (ii) ELA dosed HTT (over factorials)

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Pilot scale steam explosion reactor work

6. Scale up of XOS production from BSG in steam explosion by using the preferred and optimal process conditions from bench scale optimisations reported (Gomez *et al.*, 2015) by:
   (i) Conduct steam explosion runs at 180 ºC 10 min for preferred condition and at 200 ºC 5 min a severity of comparable severity factor of 3.65 to nonisothermal HTT for maximum XOS reported (Gomez *et al.*, 2015)
   (ii) Assess effect of screw press dewatering by using BSG at three stages of screw pressing including: raw unpressed, level A and level B pressed BSG
   (iii) Assess the effect of small differences in chemical composition by using two types of BSG, from a Weiss and malt brew.
   (iv) Assess the effect of SO_2 catalyst addition at the preferred condition by comparing XOS depolymerisation to xylotriose, xylobiose and degradation by-products formation from two SO_2 dosing methods, aqueous solution or solid as potassium metabisulfite, $K_2S_2O_5$.

7. Determine the effect of the screw press dewatering on HTT by comparing the pH, CSF and acidification in HTT (mol H+ released per dry gram of BSG) between different BSG (raw, level A, level B) with:
   (i) Establish the effect of moisture and small compositional changes with screw press dewatering in BSG on SE HTT process. Compare the SE acidification results from screw press dried BSG at optimal process condition (200 ºC and 5 min) with an air dried BSG sample.
   (ii) Determine the preferred system for high solids HTT of BSG by comparing the two types of HTT systems (Parr and SE), for HTT processing of BSG's, at the preferred process condition for acidification and degradation by-products obtained.

B. Objective #2: Techno-economic assessment of novel processes for the valorisation of BSG

1. Considering the small scale biorefinery concept co-located at a brewery and using the improved HTT processes developed through increased solids loadings obtained from screw press dewatering (Objective #1), create process flow scenarios and process simulation models in Aspen Plus® to determine mass and energy balance calculations for scenarios:

(i) for XOS production
(ii) for xylitol production and
(iii) combining both XOS and xylitol production.

2. Determine if a small scale biorefinery scenario of (i), (ii) or (iii) provides a viable financial outcome by comparing the process scenarios through detailed economic analysis to establish economic viability and identify profitable scenarios through operating cost estimation, capital cost estimation of the scenarios, and by determining minimum selling price, internal rate of return, net present value and payback period.

3. Identify important process parameters to which profitability is most sensitive

3.1.2 Research methodology

Methodology envisioned for achieving the objectives is the use of a screw press to dewater BSG, as this is reported to be the most appropriate solution (see Section **Error! Reference source not found.**). A 2.2 kW single screw press (NEW eco-tec Verfahrenstechnik GmbH, Germany), was used to reduce moisture content in the BSG. A modification was made at the outlet of the press, which allowed for a measure of backpressure adjustment, which affected the throughput and the water removal or dry mass in the solid product. Triplicate runs were conducted to assess the screw press operation. Selected random feed samples were taken of raw feedstocks and pressed products (25 and 32% dry matter).Methodology used to achieve this objective used (a) the Parr and (b) steam explosion, since these two HTT technologies are most reported reactor systems for conducting low and high solids hydrothermal tests respectively.

3.1.2.1 Parr experimental work

First, screening runs were conducted in the 1 L high pressure stirred Parr reactor with electrical jacket heating to generate BSG HTT data over a broad range of HTT process conditions, using the BSG-R (15.3% dry matter) and BSG-SPD (25% dry matter). Preferred autocatalytic HTT conditions reported for BSG hemicellulose, at 180 °C with no acid loading for maximum XOS (Gomez et al., 2015), and at 120 °C with 100 mg H_2SO_4/g dry mass loaded for maximum xylose yield (Mussatto and Roberto, 2005), were applied (Table 3-1). Rather than the low solids loadings of less than 9-11% dry matter reported before, these runs were repeated with the BSG-R (15.3% dm) and BSG-SPD (25% dm) higher solids loadings. An additional two concentrations of acid dosage, i.e. 12.5 and 46 mg acid/g dry mass loaded, were also tested at 150 °C and 120 °C. HTT process

performances were measured in terms of BSG hemicellulose solubilisation and depolymerisation, and the resulting yields of XOS, xylose and degradation products. The results were used to establish the highest level of acid loading that could be used to obtain mostly XOS for the range of conditions of the subsequent ELA HTT process optimisation through a factorial experimental design.

Table 3-1 Screen process conditions for BSG-R and BSG-SPD

Run set A for BSG-R	AH-A1	AH-A2	A-ELA	A-Z	A-DA
Run set B for BSG-SPD	AH-B1	AH-B2	B-ELA	B-Z	B-DA
Temperature (°C)	180	150	150	120	120
Acid loading (mg/g dm)	0	0	12.5	46	100
Time (min)	5*	10	10	15	15

*For BSG-R and additional run (run AH-A3) of 15 min included

The screening results found xylan solubilisation with nearly 50% xylose yield with 46 mg acid/g dry mass already; therefore, to obtain mostly XOS, the highest level of acid loading to be used in subsequent ELA HTT factorials experimental design was chosen as 20 mg acid/g dry mass for the range of conditions of temperature and time.

A full factorial design with three centre points each was done for the two different BSG start materials, i.e., BSG-R (15.3% dry matter) and BSG-SPD (25% dry matter). For each of the two processes, 11 batch runs were performed to evaluate the output variables, while the independent variables, namely, acid loadings (5-20 mg H_2SO_4/g dry mass), temperature (130-170 °C) and residence time (5-15 min), were varied to fit a 23 factorial design. The centre points were conducted in triplicate to estimate significance of curvature and the experimental reproducibility or error. A multiple regression analysis was carried out using Statistica 13.0 (StatSoft, Inc. Tulsa, USA) to describe the relationship between the independent variables for only four important selected output variables from 68 output variables recorded: XOS yield, total xylan equivalent yield, inhibitors yield (g/100 g dry matter) and total dissolved solids yield. Results were assessed with analysis of variance (ANOVA) and the degree of fit (R2) to the models was estimated. Additionally, a combined severity function was used to aid in comparing HTT results with literature and between the two different types of BSG tested.

3.1.2.2 Steam explosion experimental work

Steam explosion experiments were carried out in a pilot scale 19 l capacity “steam gun” system; a cylindrical high pressure reactor vessel and saturated steam from an electrical boiler was used to heat the material to the required temperature. An explosive flash discharge cools and releases the BSG hydrolysate slurry into a flash tank once the

required residence time has been reached. Residue was collected from the flash tank for analysis. Steam explosion treatment times and temperatures of the two BSG feedstocks are given in Table 3-2, runs A1-12 for WBSG and runs B1-7 for PBSG. SE treatment times and temperatures of the two BSG feedstocks are given in Table 5-1; for WBSG (runs A1-A12) and for PBSG (runs B1-B7). These HTT conditions used include, from Chapter 4, 180 ºC and 15 min, which showed highest XOS yields (78.0%) using WBSG (15% dry matter). Preferred process conditions, (i) 180 ºC and 10 min, was selected from reported maximum XOS + ArOS yields from multivariate optimisations in LHW HTT (11% dry matter) and (ii) 200 ºC and 5 min (SF of 3.65) was selected from reported maximum XOS + ArOS yield from nonisothermal LHW HTT optimisation in stirred batch reactors (11% dry matter) at 195 ºC (SF of 3.65). Catalysed SE HTT runs (A-11/12 and B-7) with SO_2 were conducted at process conditions for highest XOS yield obtained in uncatalysed SE. To achieve the desired SO_2 loading in the BSG, a 4.5 wt.% SO_2 was added in BSG prior to SE (runs A-11 and B-7), however in run A-12 potassium metabisulphite ($K_2S_2O_5$) was added (57% wt.% equivalent SO_2) to reach the 30 mg equivalent SO_2/g dry feedstock (Table 5-1). Air dried feedstock (WBSG-90%) was also prepared for run A-9 from WBSG-25% for SE benchmarking control run A-7.

Table 3-2 Steam explosion runs for WBSG and PBSG

BSG Type		Process Input variables		
WBSG	BSG	Starting dry mass (wt.%)	Temperature (°C)	Time (min)
A-1	B-1	15	180	10
A-2		15	180	25
A-3		25	150	25
A-4	B-2[c]	25	180	10
A-5	B-3	25	180	15
A-6		25	180	25
A-7	B-4	25	200	5
A-8	B-5	32	180	10
A-9	B-6	32	200	5
A-10[a]		90	200	5
A-11	B-7	25	180	10
25 mg SO_2	35 mg SO_2			
A-12		25	180	10
30 mg SO_2eq[b]				

Five important output variables were selected from the output variables recorded, including XOS yield, hemicellulose yield, total xylan equivalent yield, inhibitors yield (g/100 g dry matter) and total dissolved solids yield. Results were used to determine statistical significance and variable effects using Student's t-tests, which were visualised in standardised Pareto charts. Additionally, a combined severity function was calculated using the resulting pH in the slurry after steam explosion to aid in the assessment and comparison of output variables.

Gluco-, xylo-, and arabino-oligosaccharide yields (GlcOS, XOS and ArOS) after hydrothermal treatments were calculated as the weight percentage or quotient oligosaccharide recovered in the liquid hydrolysate from the starting oligosaccharide (glucan, xylan and arabinan) in the feedstock BSG. Yield of xylobiose (X2) and xylotriose (X3) was calculated as the weight percentage of the XOS in the hydrolysate. The oligosaccharide yield relative to reducing sugars yield (GlcOS%, ArOS%, and XOS%) was calculated as the mass oligosaccharide recovered, relative to total equivalent weight oligosaccharide recovered (TXeR). The total oligosaccharide equivalent weight recovered is the mass equivalent reducing sugars and oligosaccharide recovered in the hydrolysate liquid after a treatment.

Considering the literature reported above, screening runs and equipment limitations a set of ranges for independent variables were used in the experimental runs. These are set out in Table 3-3.

Table 3-3 Independent and output variables tested and reported

Independent variables	ELA aided LHW (Parr reactor)	Steam explosion	Output variables
Temperature:	**Main runs:** 120-180 °C **Screening runs:** 130-170 °C	150-200 °C	XOS yield
Treatment time:	5-15 min	5-25 min	Total xylan eq yield
Acid catalyst added: (mg/g dry matter)	**Main runs:** 5-20[a] **Screening runs:** 0-100[a]	**Main runs:** None **At highest yield:** 25-35[b]	ArOS[c], xylose, Inhibitors, TDS yield **Concentration in XOS:** Xylobiose/xylotriose
Dry matter content:	15%/25%	15%/25%/32%	
BSG type used:	WBSG	WBSG/PBSG	

[a] H_2SO_4; [b] SO_2 or $K_2S_2O_5$; [c]Arabinan-oligosaccharides

The batch Parr reactor used for ELA HTT is equipped with stirrer that is more suitable for direct acid loading for such low dosed catalysis aided LHW hydrothermal treatment, especially at the low moisture contents. Uncatalysed SE batch system is unstirred and the SO_2 catalyst addition was done only at the highest XOS yield using a 4.5 wt. % SO_2 solution or potassium metabisulfite ($K_2S_2O_5$) for an assessment on their impact on hemicellulose recovery and depolymerisation of XOS to xylobiose, xylotriose and xylose.

Apart from process variables two types of BSG were tested, (i) for their screw press performance and (ii) effect on the steam explosion hydrothermal process. The main output variable investigated was XOS yield calculated as the mass percentage of the original BSG xylan recovered in the hydrothermal treated hydrolysate. Total xylan equivalent yield include the XOS and xylose on the xylan equivalent basis as a measure of the overall

xylan recovery and efficiency of the hydrothermal process. Arabinose substitution of the XOS established separately with the yield of arabinan (ArOS) in the hydrolysate. Total dissolved solids (TDS) yield for the hydrothermal treatments were also determined to establish the concentration of fraction of XOS and ArOS in dissolved solids. Two short chain XOS (xylobiose and xylotriose) were quantified in the XOS fraction as a measure of establishing the DP of the XOS

3.1.2.3 Summary of overall work in flow diagram

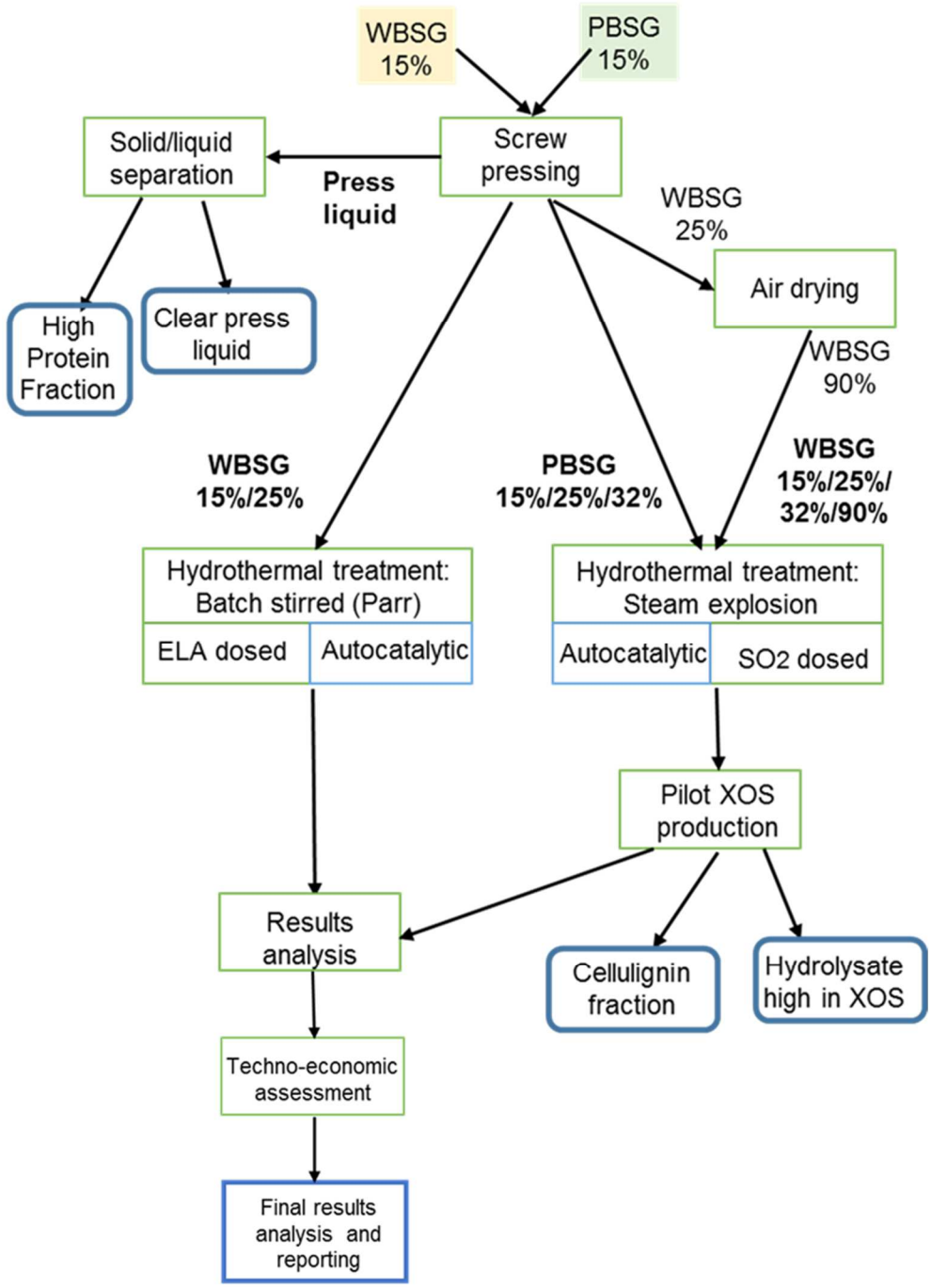

Figure 3-2 Workflow diagram for this work

3.2 References

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

Mussatto, S.I., Roberto, I.C., 2005. Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J. Sci. Food Agric. 85, 2453–2460. https://doi.org/10.1002/jsfa.2276

4. Intensification of xylo-oligosaccharides production by hydrothermal treatment of brewer's spent grains: Use of extremely low acid catalyst for reduction of degradation products associated with high solids loading

Contribution to overall scope of work

In this Chapter (Chapter 4), the first part of objective 1, involved the maximisation of XOS production from LHW HTT of brewer's spent grains (BSG) using a stirred batch reactor (Parr) in an energy efficient manner by ELA dosing with increased solids loading (>11% dm). A baseline for comparison was established by treating both raw BSG (BSG-R with 15% dm)) and screw press dewatered BSG (BSG-SPD with 25% dm) using reported optimal LHW HTT conditions for maximum XOS and xylose production. Considering the results, a range of HTT conditions were selected for multivariate optimisation using simple factorial experimental designs. The main HTT treatment response, XOS yield, assisted in characterising extremely low acid (ELA) effect on LHW HTT for the hemicellulose fractionation efficiency from the two BSG feedstocks used. Key findings include:

- Higher dry matter content (>11% dm) in LHW HTT at near optimal process conditions for XOS production (180 ºC 15 min) was shown to result in higher XOS yields (78.0%) compared to literature reported optimisations of time and temperature using 9-11% dm (190 ºC 5 min).
- A significant process intensification was achieved from current reported LHW HTT processes by using a combination of a screw press dewatering step and ELA dosing of the LHW HTT. XOS yields (61-76.4%) comparable to that previously reported for autocatalytic LHW HTT processes (61-77% 190-200 ºC) yet the results in this study were obtained at >30 ºC reduced process temperatures.The process intensification achieved a >60% reduction in water content of the BSG from the high solids loadings applied in this study.
- Contrary to commonly reported, when using higher dry matter concentrations, degradation or byproduct formation using 25% dm BSG-SPD was reduced significantly by only 5 mg H_2SO_4/g dm ELA dosing in LHW HTT (1.14 g/100 g BSG at 170 ºC 5 min) when compared to reported 9-11% dm LHW HTT (1.89 g/100 g BSG at 190 ºC 5 min) using no ELA.

Acidification in HTT of BSG and the effect of screw press dewatering:

- Screw press dewatering of BSG showed no significant effect on H+ released in LHW HTT (no acid) in the range of 150-180 ºC tested and differences in pH obtained in hydrolysates from BSG-R and BSG-SPD approximate water reduction.
- Screw press dewatering enhanced acidification in ELA dosed LHW HTT that resulted in >400% acidification, significantly more than that a water reduction.

The stirred batch reactor work from this chapter (Chapter 4) was used for pilot scale XOS production study in Chapter 5.

- The results from the high solids loadings HTT in this study (near optimal process conditions for XOS production at 180 ºC 15 min) was used to scale up in steam explosion for high solids processing.
- Results from the screw press using WBSG was also used in the Chapter 5.

The stirred batch reactor work from this chapter (Chapter 4) was used for techno-economic analysis of this novel process in Chapter 6.

- The results from the high solids loadings HTT in this study was used to compare HTT of BSG in stirred batch and the screw type reactors typically recommended for high solids processing.
- A preferred HTT process condition (46 mg H_2SO_4/g dm) was selected from the results and used in process model (Scenario C) for producing near 50/50 split in XOS and xylose from 25% dm pressed BSG.
- Hydrothermal treatment results from this preferred confdition including yields of all sugars and oligomers were used in the process model. The composition of the remaining solids after treatment were also incoprporated in the process model including protein, lignin and ash.
- Screw press component fractionation data in the pressed solid and liquid (suspended and soluble) from this chapter was used in in Chapter 6.

Intensification of xylo-oligosaccharides production by hydrothermal treatment of brewer's spent grains: Use of extremely low acid catalyst for reduction of degradation products associated with high solids loading

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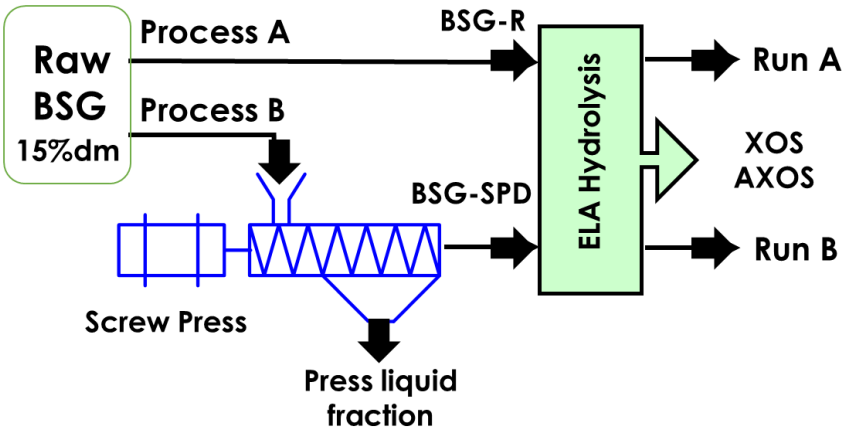
Authors' contributions

LS performed selected screening experiments and designed the experimental design, performed the batch runs and the statistical analysis. OB, EVR, and JG contributed to data interpretation and drafting of the final manuscript. All authors provided input to the manuscript and read and approved the final manuscript.

Abstract

Brewers' spent grains (BSG) make up to 85% of a brewery's solid waste, and is either sent to landfill or sold as cheap animal feed supplement. Xylo-oligosaccharides (XOS) obtained from BSG are antioxidants and prebiotics that can be used in food formulations as low calorie sweeteners and texturisers. The effect of extremely low acid (ELA) catalysis in liquid hot water (LHW) hydrothermal treatment (HTT) was assessed using BSG with dry matter contents of 15% and 25%, achieved by dewatering using a screw press. Batch experiments at low acid loadings of 5, 12.5 and 20 mg/g dry mass and temperatures of 120, 150 and 170 °C significantly affected XOS yield at both levels of dry mass considered. Maximum XOS yields of 76.4% (16.6 g/l) and 65.5% (31.7 g/l) were achieved from raw BSG and screw pressed BSG respectively, both at 170 °C and using 5 mg acid/g dry mass, after 15 min and 5 min, respectively. These XOS yields were obtained with BSG containing up to 63% less water and temperatures more than 20 °C lower than that reported previously. The finding confirms that ELA dosing in LHW HTT allows lowering of the required temperature that can result in a reduction of degradation products, which is especially relevant under high solids conditions. This substantial XOS production intensification through higher solids loadings in HTT, not only achieved high product yield, but also provided benefits such as increased product concentrations and decreased process heat requirements.

Keywords: Brewers' spent grains; xylo-oligosaccharides; extremely low acid; screw press drying; high solids loading



Highlights

- Simple screw pressing effective for brewers' spent grains water removal and conditioning
- High solid loading (15-25% dm) HTT of brewers' spent grains for hemicellulose solubilisation
- Extremely low acid <0.7 wt.% (5-20 mg/g dry mass) catalysed xylan depolymerisation and solubilisation for XOS, xylotriose, xylobiose and xylose production

4.1 Introduction

Breweries produce around 40 million tonnes of brewers' spent grains (BSG) annually, which constitutes up to 85% of the solid waste produced by a brewery (Mussatto, Dragone and Roberto, 2006). Due to its high moisture content (~70-85% water) and bioactive organic matter, BSG waste is highly susceptible to decomposing (Robertson, lAnson, *et al.*, 2010; Wang *et al.*, 2014), and is either used for animal feed or consigned to landfill (Rosa and Beloborodko, 2015; Kerby and Vriesekoop, 2017). However, stricter regulations on animal feed and a drive to improve resource efficiency have compelled breweries to find alternative applications for BSG (Thomas, K. R.; Rahman, 2006; Kerby and Vriesekoop, 2017).

Various alternative applications of BSG have been reported, including conversion to biogas, bio-oil, carboxylic acid, levulinic acid, biobutanol and xylitol (Skendi, Harasym and Galanakis, 2018). However, BSG from brewing is a food-grade lignocellulosic by-product that has potential for high value food product applications. BSG contains a wide range of health-promoting compounds, nutraceuticals and phytochemicals, which can be extracted and reapplied in food and beverage formulations (Lynch, Steffen and Arendt, 2016; Steiner, 2016; Abu-ghannam and Balboa, 2018). The relatively large (>25%) hemicellulose fraction, mainly xylan and arabinan, makes BSG well suited as a raw material for producing health promoting dietary fibres or prebiotics, in particular xylo-oligosaccharides (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). The degree of polymerisation (DP) of XOS is reported to be an important factor affecting the biological properties thereof, with short-chain XOS (DP<10) having the greater bifidogenic or prebiotic effect (Moura, Carvalheiro, Esteves, 2008). Furthermore, short-chain XOS substituted with arabinose, known as arabino-xylo-oligosaccharides (AXOS), in particular, have been found to be highly fermentable *in vitro* by beneficial Bifidobacterium of the human gut (Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). These XOS dietary fibres are found in functional food products as low-calorie sweeteners, anti-oxidants, prebiotics and texturisers, and are also marketed as supplements.

Efficient fractionation of XOS from BSG requires selective solubilisation of the hemicellulose-xylan component, which can be achieved with hydrothermal treatment (HTT) technologies, which have been applied to various lignocellulosic biomasses, including BSG (Yang and Wyman, 2008; Galbe and Zacchi, 2012). Current processes for oligosaccharide production from BSG hemicellulose rely on autocatalytic liquid hot water (LHW) HTT, pressurised water at elevated temperatures (120 - 250 °C), which achieves solubilisation and partial depolymerisation of xylan by hydrolysis i.e. the catalytic action of water (Cardenas-toro *et al.*, 2014). The rate of solubilisation and depolymerisation of xylan is proportional to H+

concentration and which is increased by acetic acid released by hydrolysis of acetyl groups in xylan structures, thereby creating the autocatalytic effect (Mosier, Ladisch and Ladisch, 2002; Negahdar, Delidovich and Palkovits, 2016). Autocatalytic LHW HTT of BSG reported maximum XOS yields ranging between 61% and 77% around 195 to 200 °C, yet to produce more of the preferred arabinose-substituted XOS (AXOS) temperatures below 180 °C are required (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015; López-Linares *et al.*, 2019). However, rates of solubilisation and depolymerisation are lowered at such reduced temperatures (<180 ºC), resulting in lowered (<70%) XOS yields (Gómez et al., 2015).

Additionally the LHW HTT technologies reported for XOS production from BSG use very dilute solids concentration < 11%, yet such processing use large amounts of water that is inherently very energy intensive (Leibbrandt, Knoetze and Görgens, 2011; Galbe and Zacchi, 2012; Modenbach and Nokes, 2012). Using higher solids loading (>15% dm) generally causes viscosity-related processing issues in conventional, stirred vessels (Yang and Tucker, 2013). The limitations in heat and mass transfer associated with the high solids loading typically have a negative effect on desired product yields and/or qualities (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Modenbach and Nokes, 2012; Sui and Chen, 2015) and combined pose a risk in lignocellulosic HTT of producing more degradation products such as furfural, 5-HMF and lignin fragments (Jönsson and Martín, 2016). However, higher solids loading could obtain higher product concentrations, reduced energy requirements, and reduced process equipment size (Leibbrandt, Knoetze and Görgens, 2011; Galbe and Zacchi, 2012; Modenbach and Nokes, 2012). Moreover, even higher product yields can be obtained using higher solids loadings in LHW HTT if the negative effects of high solids loading can be mitigated (Vallejos *et al.*, 2012).It is anticipated that the use of ELA dosing in LHW HTT can perhaps achieve this.

For most lignocelluloses, in the HTT supplementing the autocatalytic LHW HTT with extremely low acid (ELA) concentrations, i.e. <0.7 wt. % H_2SO_4 could increase rates and improve product yields at lower temperatures (Yang and Wyman, 2008; Shen and Wyman, 2011; Gurgel *et al.*, 2012; Kapoor *et al.*, 2017). LHW HTT with ELA is preferable since (i) the lower temperatures may achieve similar product yields as autocatalyzed LHW HTT at higher temperatures, and (ii) may reduce the extent of lignocellulose degradation into by-products (Yang and Wyman, 2008; Gurgel *et al.*, 2012). Therefore, the ELA catalysis of LHW HTT may enable the XOS production at higher solids loadings with acceptable yields, by lowering degradation by-product formation, the inherent negative effect accompanying the high solids loading. Combining high-solids and ELA-HTT technology may have environmental benefits such as reduced energy usage and waste production, to balance the environmental consequences which may be associated with minimal amount of catalyst used (<0.7 wt. %) (Yang and

Wyman, 2008; Gurgel *et al.*, 2012). Yet no such ELA dosed and high solid LHW studies on XOS production of BSG are reported.

Given the high water content of BSG (~70-85% water), increasing the solids loading implies the inclusion of a dehydration step preceding the HTT process. Mechanical dewatering is a preferred alternative to thermal dehydration, to reduce process costs (Huige, 1994). The mechanical dewatering of BSG with a screw press could reduce the water content to a minimum of about 55% (Finley, Walker and Hautala, 1976; Huige, 1994; Weger *et al.*, 2017). Screw pressing of biomass is also known to provide a degree of mechanical defibrillation and shortening of the fibres, which may facilitate the HTT (Yan *et al.*, 2014). On the other hand, the liquid fraction separated from the BSG by screw press also contains suspended solids that are high in protein (>50%) (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977), which creates a value added co-product. Given the selective removal of protein from the BSG, it is anticipated that it would be advantageous for the HTT step of the BSG. The reduced protein content in the substrate can increase the rate of depolymerisation of hemicellulose by reducing buffering capacity during the HTT (Liao *et al.*, 2004) and can result in increased oligomer and sugar yields by reduced polysaccharide-protein reaction (Rommi *et al.*, 2018)

It is anticipated that applying a mechanical dewatering of BSG through a screw press, to achieve high solids loadings for HTT, and combining this with ELA catalysis, may provide an opportunity to significantly improve the process for XOS production from BSG with some technical and economic advantage. Therefore, this study investigated the effect of higher solids loading achieved by mechanical dewatering of BSG through screw pressing on XOS production from BSG. Using two BSG's obtained, raw and pressed, the ELA catalysis of LHW HTT was optimised for XOS production in a stirred batch Parr reactor system to reduce acid use, required process temperatures and reduce the degradation product formation (acetic acid, formic acid, furfural and HMF). Firstly, reported optimised process conditions for XOS production in autocatalytic LHW HTT (9/11% dm) were applied in the batch Parr reactor system with the BSG feedstocks (15/25% dm) and used as a benchmark for the ELA catalysed LHW HTT. Investigated process conditions for the ELA catalysed HTT were H_2SO_4 dosing at (5, 12.5 and 20 mg/g dry mass) and temperatures (130, 150 and 170 °C), using dry matter contents of 15% (BSG-R) and 25% (BSG-SPD), achieved with screw press dewatering. The BSG hemicellulose solubilisation products XOS, ArOS (Arabinan in XOS), xylobiose, xylotriose and xylose yields and the degradation products were compared under these conditions. A combined severity factor (CSF) was used to evaluate results from the autocatalytic HTT and ELA catalyst HTT treatments on the BSG-R and BSG-SPD. The results show process conditions for maximisation of XOS and minimisation of degradation products at high solids loading from BSG in LHW HTT by use of ELA dosing. The high XOS yields

obtained even with the high solids loading of BSG with the ELA catalysed LHW HTT provides insight for improved production of XOS.

4.2 Materials and methods

4.2.1 Raw material and screw press drying

Fresh BSG was obtained as a 1 m^3 batch from a local brewery (Newlands, Cape Town, South Africa); it consisted of a single brew from a Weiss recipe with a 50:50 ratio of malted barley and wheat. A continuous screw press (NEW Eco-tec Verfahrenstechnik GmbH, Mühldorf, Germany) with a 0.3 m long x 0.15 m diameter screen cage and driven by a 2.2 kW 3-phase motor was used to press 400 kg of BSG for dewatering. Samples of the raw BSG (BSG-R) and pressed BSG (BSG-SPD) were aliquoted in sealed vacuum bags and stored frozen at -20 °C. Samples were thawed in a 25 °C water bath before use.

4.2.2 Batch processing equipment

A Hastalloy C-276 model 4540 high pressure 1 litre bench reactor from Parr Instruments Company (Moline, Illinois, USA) was used for acid catalysed LHW HTT of BSG-R and BSG-SPD. Mixing inside the reactor was controlled with a variable speed motor driving through a magnetic coupling to an impeller with twin six-blade Rushton-type impellers. An external electric band heater was controlled by a proportional-integral-derivative (PID) controller, model 4842 (Parr). An operational procedure described by Carvalheiro et al.,(2005b) and Duarte et al., (2004) was used, with slight modifications for wet BSG without thermal drying and the acid dosing. Direct acid dosing of the wet BSG was applied in the reactor, instead of soaking dry material, as suggested by Galbe and Zacchi (2012). The BSG was loaded into the reactor, and during mixing the acid was dosed directly to obtain the required acid loading of between 5 and 20 mg/g dry mass loaded. The agitation rate during reactions was adjusted to 40 rpm. After desired treatment time the reactor contents were recovered and filtered to obtain liquid for analysis.

4.2.2.1 Prefactorial screening experiments

Firstly, the screening runs were conducted to define an acceptable range of process conditions for the ELA catalysed LHW HTT conducted on the BSG-R (15.3% dm) and BSG-SPD (25% dm). The autocatalyzed LHW HTT runs in the screening experiments provided a benchmark for the ELA catalysed LHW HTT results obtained in this study. Preferred autocatalytic HTT conditions reported for BSG hemicellulose, at 180 °C with no acid loading for maximum XOS (Gomez *et al.*, 2015), and at 120 °C with 100 mg H_2SO_4/g dry mass loaded for maximum xylose yield (Mussatto and Roberto, 2005), were applied (Table 4.1). Rather than the low solids loadings of less than 9-11% dry matter reported before, these runs were repeated with

the BSG-R (15.3% dm) and BSG-SPD (25% dm) higher solids loadings. An additional two concentrations of acid dosage, i.e. 12.5 and 46 mg acid/g dry mass loaded, were also tested at 150 °C and 120 °C. HTT process performances were measured in terms of BSG hemicellulose solubilisation and depolymerisation, and the resulting yields of XOS, xylose and degradation products. The results were used to establish the highest level of acid loading that could be used to obtain mostly XOS for the range of conditions of the subsequent ELA HTT process optimisation through a factorial experimental design.

Table 4-1 Screen process conditions for BSG-R and BSG-SPD

Run set A for BSG-R	AH-A1	AH-A2	A-ELA	A-Z	A-DA
Run set B for BSG-SPD	AH-B1	AH-B2	B-ELA	B-Z	B-DA
Temperature (°C)	180	150	150	120	120
Acid loading (mg/g dm)	0	0	12.5	46	100
Time (min)	5*	10	10	15	15

*For BSG-R and additional run (run AH-A3) of 15 min included

4.2.2.2 ELA factorial experimental design

A full factorial design with three center points was conducted separately for each of the two different BSG feedstocks, i.e. BSG-R and BSG-SPD. For each feedstock, 11 batch runs (A-1 to 11 and B1 to 11) were performed to evaluate the output variables, while independent variables, namely, acid loadings, temperature and residence time, were varied to fit a 2^3 factorial design (Table 4.2). Ranges for the values for the variables were selected from literature and from the results of the pre-factorial screening experiments as described in the previous section.

Table 4-2 Experimental factorial design for independent variables

Independent variables		**Range and levels**		
		-1	0	+1
Acid loading (mg/g dm)[a]	AC	5	12.5	20
Time (min)	t	5	10	15
Temperature (°C)	T	130	150	170

[a] Dry mass of feedstock material loaded

The factorial experimental design runs, A1–A11 and B1-11 for BSG-R and BSG-SPD respectively, were done independently. In the experimental design, the high, mid and low points were coded -1, 0 and 1 respectively (Table 4.2). The center points were done in triplicate to estimate significance of curvature and the experimental reproducibility or error.

A multiple regression analysis was carried out using STATISTICA 13.0 (StatSoft, Inc. Tulsa, USA) to establish the coefficients in eq.1 to describe the relationship between the independent

variables for four output variables or responses: XOS yield, xylan equivalent yield inhibitors yield (g/100g dm) and total dissolved solids (TDS) yield. Models were constructed to fit the data according to the following equation for Y, the predicted response for the independent variable:

$$Y = a_0+a_1AC+a_2t+a_3T+a_{12}ACt+a_{13}ACT+a_{23}tT+a_{123}ACtT \qquad \text{eq.1}$$

The coefficients, a_{ij} are adjustable constants optimised for the model fit and the statistical significance of each was determined ($p<0.05$). Results were assessed with analysis of variance (ANOVA) and the degree of fit (R^2) to the models was estimated.

Additionally, a combined severity function (CSF) was used to aid in the comparison of HTT results in this study with that from literature and between the two BSG feedstocks tested. The autocatalytic HTT severity function for combining contribution of time (t) and temperature (T_r) during HTT, was used in the adapted form for the incorporation of the acid catalyst applied with CSF = log R_0-pH; where $R_0 = t .\exp((T_r -100)/14.75)$ (Chum *et al.*, 1990). Apart from using the resulting pH in the CSF calculation, the resulting pH, converted to mol H+ per gram dry BSG, was also used to compare hydrolysate acidification in the HTT. The resulting ratio, R[H+], of mol H+ per gram dry BSG-SPD relative to mol H+ per gram dry BSG-R, was used for comparison of the HTT results between the two ELA catalysed LHW HTT factorial sets run A vs run B, and the autocatalytic LHW HTT runs from the pre-factorial screening.

4.2.3 Analytical methods

Standard Laboratory Analytical Procedures (LAPs) of the National Renewable Energy Laboratory (NREL, USA) were used for biomass compositional analysis (Sluiter et al. 2010). A starch kit from Megazyme (K-TSTA, Ireland) was used to determine total and residual starch after ethanol wash of the BSG samples (Robertson et al. 2010). Amino acids were determined using a Waters Acquity (Milford, USA) Ultra Performance Liquid Chromatograph (UPLC) separation with ultraviolet (UV) or fluorescence detection after derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). The crude protein content of samples, based on the nitrogen content of protein, was determined using Kjeldahl analysis (DK8 Velp Scientifica, Usmate, Italy), using a factor of N x 6.25 (Pires et al. 2012). All samples were analysed, at least, in triplicate.

The concentrations of short-chain oligomers (xylotriose, xylobiose), sugars (glucose, xylose and arabinose) and degradation products (acetic and formic acid, hydroxymethylfurfural (HMF) and furfural) were analysed by high performance liquid chromatography (HPLC) on a Aminex HPX-87H Ion Exclusion Column equipped with a Cation-H cartridge (Biorad, Johannesburg, South Arica) (García-Aparicio *et al.*, 2011). Samples from the liquid fraction after HTT were filtered through a 0.2 µm filter before analysis.

To quantify oligosaccharides (GlcOS- glucooligosacharides, XOS and ArOS) in the hydrolysate, liquid samples were subjected to posthydrolysis using 4% wt. H_2SO_4 at 121 °C for 40 min (Sluiter *et al.*, 2010). Oligosaccharides, which included soluble polymers and oligomers, were defined as the difference in the monomeric sugar concentration before and after posthydrolysis of the filtered hydrolysate (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015).

The oligosaccharide (XOS and ArOS) yields were defined as the mass fractions (%) of the initial xylan and arabinan in the dry mass feedstock that was recovered in the hydrolysate liquid after treatment (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). The total polysaccharide equivalent (TX_eR) weight recovered was calculated by the total mass equivalent of reducing sugars and oligosaccharide recovered in the liquid from the starting polysaccharide after a treatment. The oligosaccharide yield relative to reducing sugars yield (ArOS%, and XOS%) was calculated as the mass oligosaccharide recovered, relative to total equivalent weight polysaccharide recovered.

4.3 Results and discussion

4.3.1 Screw press dewatering of BSG

The dry matter content of the BSG-R (15.3%) was increased by means of the mechanical dewatering using a screw press, resulting in the BSG-SPD with dry matter content of 25%. This was achieved at a throughput rate of 794 kg BSG-R per hour. However, the screw press treatment in this study was relatively moderate, since the maximum of 25% dry matter content obtained in the present study (Figure 4-1), was below the 35-40% dry matter reported (Weger *et al.*, 2014; Weber and Stadlbauer, 2017). However, the energy requirements reported (40-53 kWh/t) for achieving that high solids content (Weger *et al.*, 2014) is nearly 20 times higher than that used in this study per tonne of wet BSG (2.8 kWh/t = 2.2 kW/0.794 t/h). This is a fraction of the 270-320 kWh/t estimated energy requirements for drying by steam to achieve the same moisture reduction (Huige, 1994). A mass balance revealed that 38.6% of the water fraction from BSG-R and 71.3% of the dry matter was recovered in the BSG-SPD, while the remainder was removed in the press liquid stream (Figure 4-1). A considerable reduction in the dissolved solids (DS) fraction of total solids (dry mass) was achieved as a result of the water removal. The dissolved solid fraction in BSG-R reduced from 10.8% (1.6/15.3) to 5.8% (1.5/25) in the BSG-SPD (Figure 4-1).

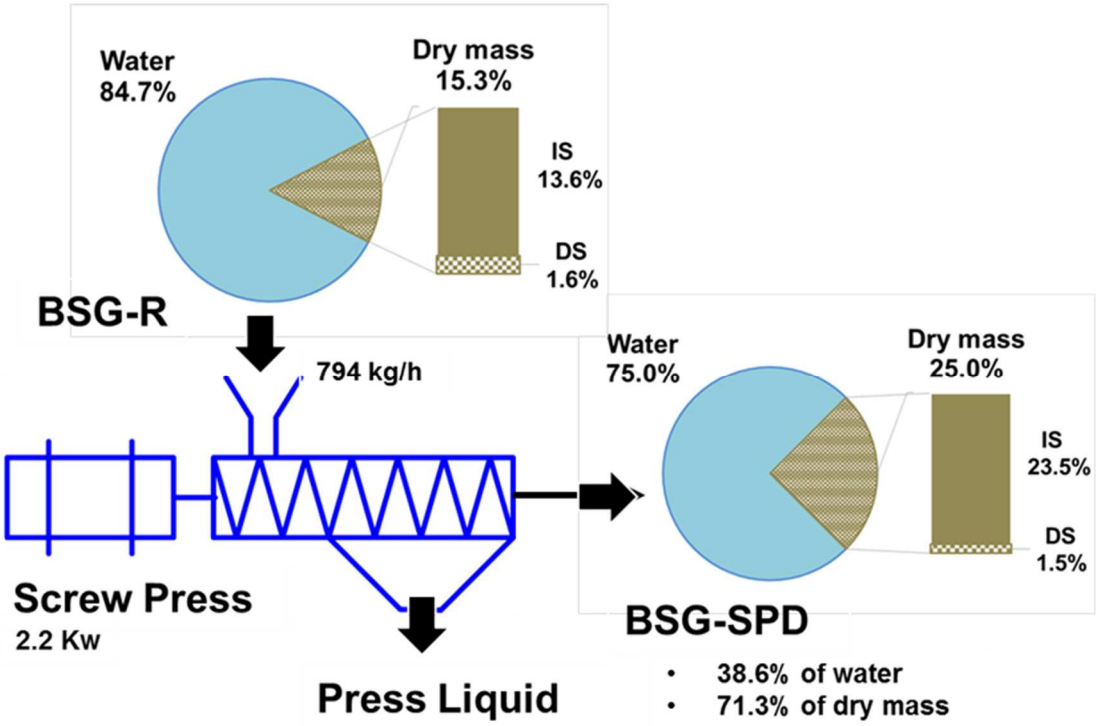


Figure 4-1 Water and dry matter balance over screw press dewatering operation: Resulting soluble and insoluble split (DS- dissolved solids, IS Insoluble solids)

A benefit of screw press dewatering is the selective removal of components such as starch and protein from the BSG-R into the separated press liquid fraction (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Jay *et al.*, 2008). The screw press dewatering process resulted in the removal of soluble components and suspended materials in the liquid press fraction. The total starch content was reduced from 12.9 to 9.2%, the crude protein content also decreased from 24.3% to 21.8% (Table 4.3). Consequently, this translated into 49.1% and 36.0% selective removal of starch and protein from the BSG-R into the press liquid fraction. These findings were consistent with the selective removal of fine BSG particles (<150 µm) with mechanical screw press dewatering that are reported to be high in starch and protein (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Jay *et al.*, 2008). Due to the selective removal of proteins and starch by the screw press dewatering process, the BSG contents of cellulose and hemicellulose increased from 10.4% and 18.9%, respectively, in the BSG-R, to 11.8 and 21.8%, respectively, in the BSG-SPD. The increase in fibre fraction and reduction in the interfering components (starch, proteins) of the BSG-SPD may provide a benefits to the subsequent HTT, to counteract the anticipated limitations that may occur as a result of the increased dry matter content. The compositional results of both BSG-R and BSG-SPD (Table 4.3) are consistent with other compositional values for BSG's reported by Lynch et al., (2016), for hemicellulose 19.2-41.9%, for cellulose 0.3-33%, for starch 1-12%, for protein 14.2-31%, for lignin 11.5-27.8%, for lipids 3-13% and 1.1-13% for ash content.

Table 4-3 Chemical composition of BSG-R and BSG-SPD

Components	BSG-R		BSG-SPD	
	% w/w	±SD	% w/w	±SD
Dry mass	**15.3**	*1.95*	**25.0**	*0.51*
pH	**4.96**			
Bulk density (dry basis)	**0.15**	*0.01*	**0.12**	*0.01*
Extractives	**25.6**		**21.5**	
NREL Water	13.9	*0.44*	10.1	*0.13*
NREL Ethanol	11.7	*0.14*	11.4	*0.09*
Water 25 °C	11.5	*0.57*	8.8	*0.96*
Total starch	**12.9**		**9.2**	
Starch	11.4	*0.17*	8.1	*0.53*
Maltodextrins	1.5	*0.13*	1.2	*0.10*
Cellulose	**10.4**	*0.42*	**11.8**	*0.23*
Hemicellulose	**18.9**		**21.8**	
Xylan	11.7	*0.10*	14.5	*0.05*
Arabinan	5.9	*0.21*	6.5	*0.17*
Acetyl groups	1.2	*0.00*	0.7	*0.20*
Lignin	**18.8**		**21.0**	
Acid soluble (AS)	5.3	*0.12*	6.7	*0.44*
Acid insoluble (AI)[a]	13.4	*0.06*	14.3	*0.03*
Crude protein	**24.3**	*0.35*	**21.8**	*0.65*
Ash	**2.9**	*0.01*	**3.1**	*0.02*
Total[b]	**100.9**		**102.4**	

Data shown is the mean ± standard deviation of three replicates
[a] Acid insoluble lignin corrected for ash and protein
[b] Corrected for starch and protein accounted for in NREL extractives

GlcOS of BSG (including soluble starch, maltodextrins and cellubioise) made up the largest fraction (39.6%) of the dissolved solids in the BSG liquid fraction (Table 4.4). The starch content of the liquid fraction accounted for 30.5% of the total starch in the BSG-R, which was reduced to 20.0% for the BSG-SPD owing to the screw press dewatering. Similarly, the soluble nitrogen fraction of the liquid fraction of the BSG reduced from 14.8% in the BSG-R to 9.1% in BSG-SPD. The pressed liquid from the BSG-SPD contained suspended solid products of which proteins and starch constituted the largest fractions (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977; Jay *et al.*, 2008). Interestingly the amino acids analysis of the insoluble solid showed a slightly higher content than the crude protein analysis, probably due to the under reporting of the assumed nitrogen conversion factor of 6.25 for this fraction (Table 4.4).

Table 4-4 Fraction of component as dissolved solid (DS) of BSG-R and BSG-SPD and the insoluble solid fraction in the press liquid

BSG components	Dissolved solids (DS) in BSG-R	Dissolved solids (DS) in BSG-SPD	Insoluble solid from press liquid[a]
Water ratio to dry matter	5.5	3.0	15.5
Total starch	30.5%	20.0%	22.0%
Xylan	2.0%	0.8%	3.2%
Arabinan	0.6%	0.3%	1.4%
Acetyl groups	11.8%	14.2%	n.d.
Crude protein[a]	14.8%	9.1%	35.5%
Amino acids	n.d.	n.d.	43.0%

[a] basis total IS dry matter; [b] N x 6.25

4.3.2 Pre-factorial screening experimental results

The pre-factorial screening performed showed that high solids loadings in LHW HTT of BSG can lead to higher XOS and xylose product yields and the ELA addition in LHW HTT can result in a reduction in degradation product formation and thereby improving XOS production. Additionally from the results a preferred range of process conditions was selected for the subsequent full factorial optimisation of the ELA catalysed LHW HTT for XOS production. Figure 4-2 provides the product yields and the degradation product composition of the hydrolysate under the screening conditions for the BSG-R and the BSG-SPD.

4.3.2.1 Increased solids loadings of BSG in HTT lead to higher products yields

High solids loadings can lead to higher product yields compared to dilute LHW HTT. Results from screening experiments (Figure 4-2) show the highest XOS yields of up to 78.0% (run AH-A3 using 180 ºC and 15 min) was obtained with the autocatalytic (no acid added) LHW HTT using solids loadings, of 15 % dry matter content, significantly higher compared to using dilute 9-11% dry matter content in reported LHW HTT optimisation studies with maximum XOS yields of 61% (190 ºC and 5 min). Additionally, results show increasing solids loadings in LHW HTT from 15% to 25% dm using the same process conditions (180 ºC and 5 min) maintained similar XOS yields, of 65.0% (run AH-A2) and 65.3% (run AH-B2) when using 15% dry matter content with BSG-R and 25% dm BSG-SPD respectively (Figure 4-2 A.1 and B.1). Moreover, xylose yields obtained with the dilute acid (100 mg H_2SO_4/ g dm) HTT of BSG at the optimal condition reported also increased significantly from 57.3% (run DA-A) to 71.8% (run DA-B) with increasing solids loadings from using BSG-R and BSG-SPD. These similar and higher yields were obtained with even higher xylan content in the BSG-SPD hemicellulose as a result of the screw press dewatering (Table 4-2), which implies increased solubilisation rates.

The results of higher solids loading leading to higher yields is in agreement to reported LHW HTT treatment of bagasse that showed a positive relationship of XOS yield and dry matter content at certain process conditions (Vallejos *et al.*, 2012).Therefore in LHW HTT optimisations, the solids loading (or dry matter content) is an important variable to consider in addition to temperature and time. The results in this study from the increased solids loadings (15 to 25% dm) used in XOS production by HTT of BSG, represents a significant HTT technology improvement, since these results were obtained using up to 60% less water. If the negative effects from higher solids loadings can be mitigated, increased dry matter content can lead to a significant HTT process intensification with higher product concentrations and lower water requirements that can result in reduced heat requirements. The ELA dosing is considered as a possible further improvement in LHW HTT in the factorial runs.

4.3.2.2 ELA dosing in LHW HTT of BSG for a reduction of degradation products

As can be seen in formation of degradation products, with the Figure 4-2 A.2 and B.2, runs with BSG-SPD, with the highest solids loading, overall produced more degradation products compared with BSG-R. While, for both BSG's, at lower temperatures (<150 ºC), combined with using higher acid dosing (from 46 mg H_2SO_4/g dm), resulted in more acetic acid formation, while the higher temperatures (>150 ºC) favoured formic acid and furfural. Higher acid loadings (from 46 mg H_2SO_4/ g dm), for both BSG's, accelerated depolymerisation of XOS to the monomeric xylose sugar which for BSG-SPD, in run DA-B, with the highest acid loading (100 mg H_2SO_4/ g dm) resulted in the highest amounts of xylose (11.9 g/100 g BSG) and lowest yield of XOS (0.98 g/100 g BSG) with the Figure 4-2 A.1 and B.1.

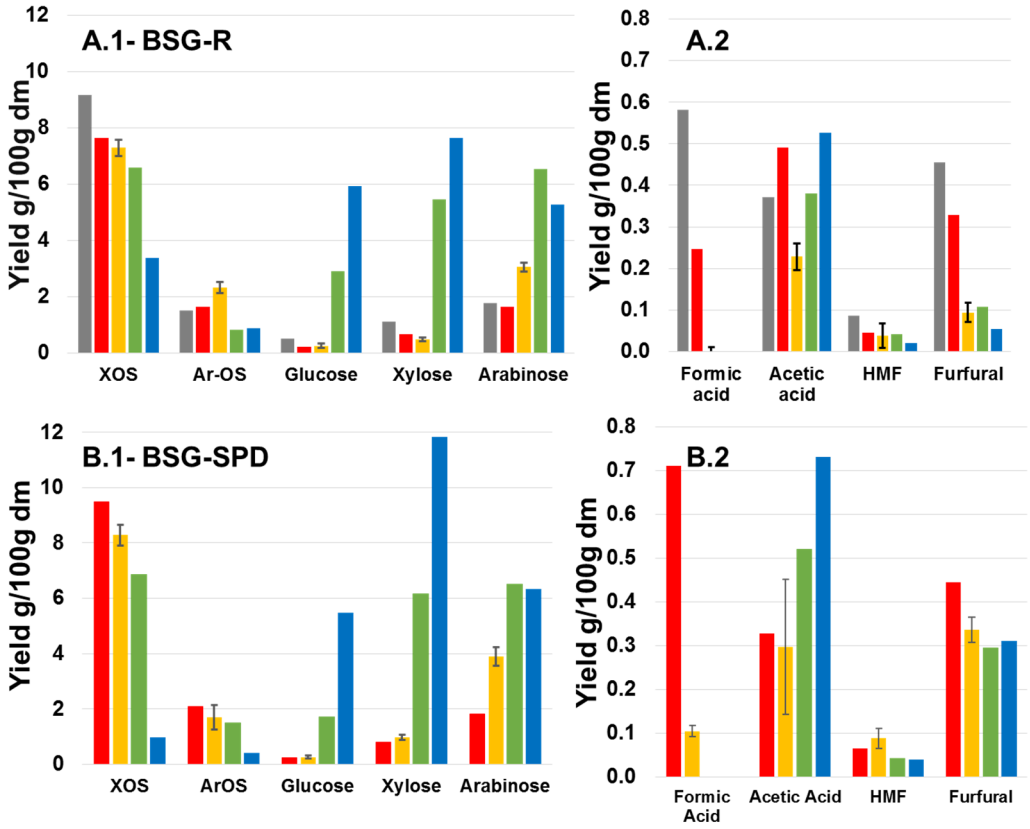


Figure 4-2 Screening runs yields (g/100 g dm) from (A) BSG-R and (B) BSG-SPD: (1) Sugar yields and (2) Degradation by-products yields: [ ] 180 °C 15 min no acid, [ ] 180 °C 5 min no acid, [ ] 150 °C 10 min 12.5 mg H_2SO_4/g dm, [ ] 120 °C 15 min 46 mg H_2SO_4/g dm, [ ] 120 °C 15 min 100 mg H_2SO_4/g dm (XOS- xylo-oligosaccharide, ArOS – Arabino-oligosaccharide, HMF – 5-Hydroxymethyl furfural)

On the other hand, with no acid, the autocatalytic LHW HTT of BSG-SPD at 180 ºC (run AH-B2) resulted in highest XOS yield (9.48 g/100 g BSG equivalent to 65.3%), however the associated degradation products found were significant (1.49 g/100 g BSG). partly due to the elevated temperatures (>180 ºC). These degradation products may present a challenge during XOS product purification. However the screening results (run ELA-A) show the ELA catalysed

LHW HTT (12.5 mg H_2SO_4/g dm at 150 ºC for BSG-R) allows for high XOS yields (61.5%) to be obtained with reduced degradation (0.36 g/100 g dry BSG) products, and at lower temperatures (<180 ºC).Therefore a final range of ELA LHW HTT conditions considered for the factorial experimental design was (i) for temperatures, between 130 and 170 °C; (ii) ELA acid loadings, 5-20 mg H_2SO_4/g dry BSG loaded; and (iii) time, 5-15 min (Table 4-2). These factorial experiments conducted were 11 batch runs each, on the BSG-R (run A-1 to 11) and BSG-SPD (run B-1 to 11) samples, with 15% and 25% dm content respectively.

4.3.3 Full-factorial optimisation of ELA LHW HTT with BSG-R and BSG-SPD for XOS production

ELA dosing of H_2SO_4 in autocatalytic LHW HTT enabled improved hemicellulose solubilisation and hemicellulose product recovery at lower temperatures. Results from the ELA HTT factorial runs A1-11 and B1-11 (Appendix Table 4A-2 and Table 4A-3) were analysed statistically and models were created to determine the significance of the investigated parameters and their optimal ranges for each output variable (XOS yield, degradation product formation, XOS concentration and TDS solids) using STATISTICA 13.0 (Appendix Table 4A-4, 4A-5 and 4A-6). An ANOVA on the results from the factorial runs revealed that the effect of acid loading and interactions were significant for XOS yield with a p-value below 0.05 for both BSG-R and BSG-SPD (Appendix Figure 4A-3 A and B). Temperature showed the most significant effect for XOS yield from the BSG-R, while the interaction of acid loading with temperature was the most significant for BSG-SPD.

4.3.3.1 ELA dosing in LHW HTT of BSG for improved XOS yields

ELA dosing in LHW HTT of BSG enables high XOS yields (>70%) at high solids loadings (>15% dm). The ELA catalysis of LHW HTT significantly improved the HTT process through increased hemicellulose solubilisation and hemicellulose product recovery at lowered process temperatures compared to the autocatalyzed LHW HTT for both BSG-R and BSG-SPD (Figure 4-3 A and B). The process intensification of ELA addition in LHW HTT of BSG at high solids loading (dry matter concentration >15% dm) through the screw press dewatering process, resulted similar or even higher XOS yields compared to reported autocatalytic LHW HTT in literature (using 9-11% dry matter content). The ELA catalysed HTT of BSG with high solids loadings in this study obtained maximum XOS yields of 76.4% (run A-4), and 65.5% (run B-2) at 170 °C for the BSG-R (Figure 4-3 A) and BSG-SPD (Figure 4-3 B) respectively. The XOS yields obtained with ELA catalysed LHW HTT are important compared to the 61% (190 ºC) and 77% (200 ºC) reported for optimised XOS production from BSG (autocatalytic LHW HTT) with lower solid loadings of 9-11% dm and even at 30 ºC higher temperatures (Carvalheiro *et al.*, 2004; Vegas *et al.*, 2005; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015;

López-Linares *et al.*, 2019). The ELA catalysis of LHW HTT significantly improved the fractionation of BSG hemicellulose, XOS and xylose yield

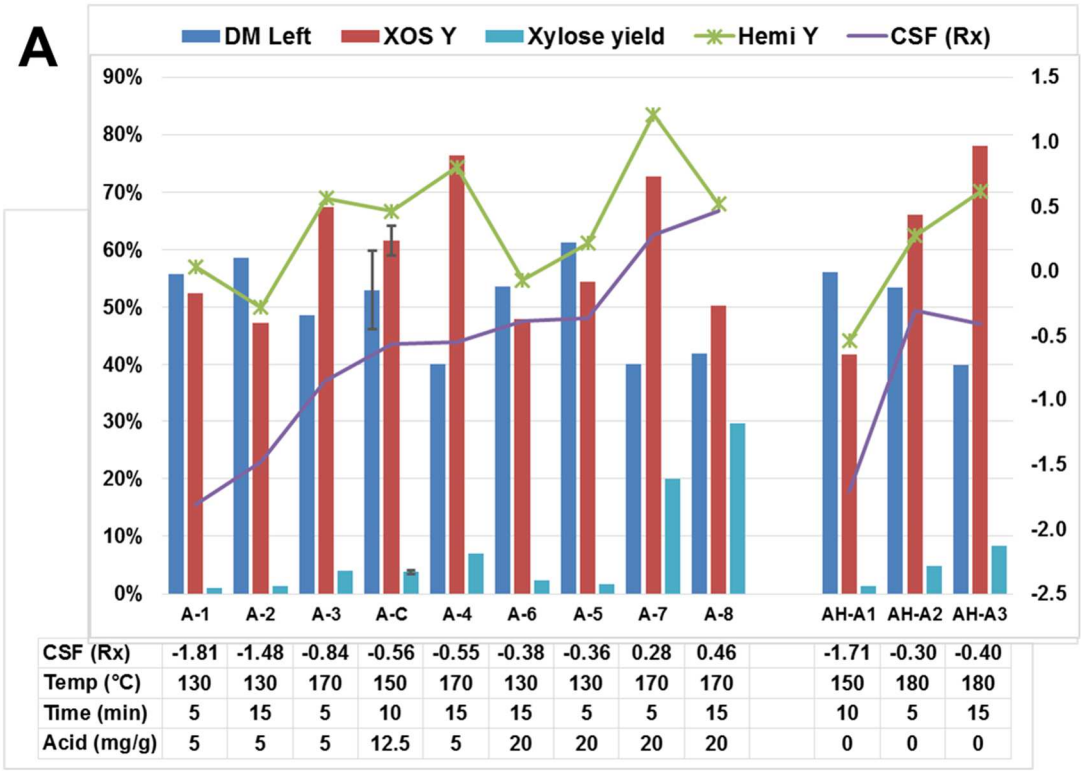
4.3.3.2 ELA dosing in LHW HTT of BSG for the reduction in degradation product formation

The undesired increased production of degradation products commonly accompanying in with increased solids loading as seen in the autocatalyzed LHW HTT can be reduced by the ELA catalysed LHW HTT process. Degradation products formed at the conditions for maximum XOS yield from the BSG-SPD (run B-3) was 1.14 g/100 g dm, which was higher than the 0.71 g/100 g dm produced from the BSG-R at the same conditions (run A-3) as shown in Table 4-5. Nevertheless, these degradation product amounts obtained with ELA dosing in LHW HTT are still significantly lower than (1.49 g/100 g dm) that produced when using no acid at 180 °C and 5 min (AH-B2). Therefore, the ELA HTT results show a substantial process intensification of the HTT technology in hemicellulose fractionation for XOS production from BSG since similar or higher yields were obtained using at least 20 °C lower temperatures, at least 20% lower degradation product formation and up to 60% less water.

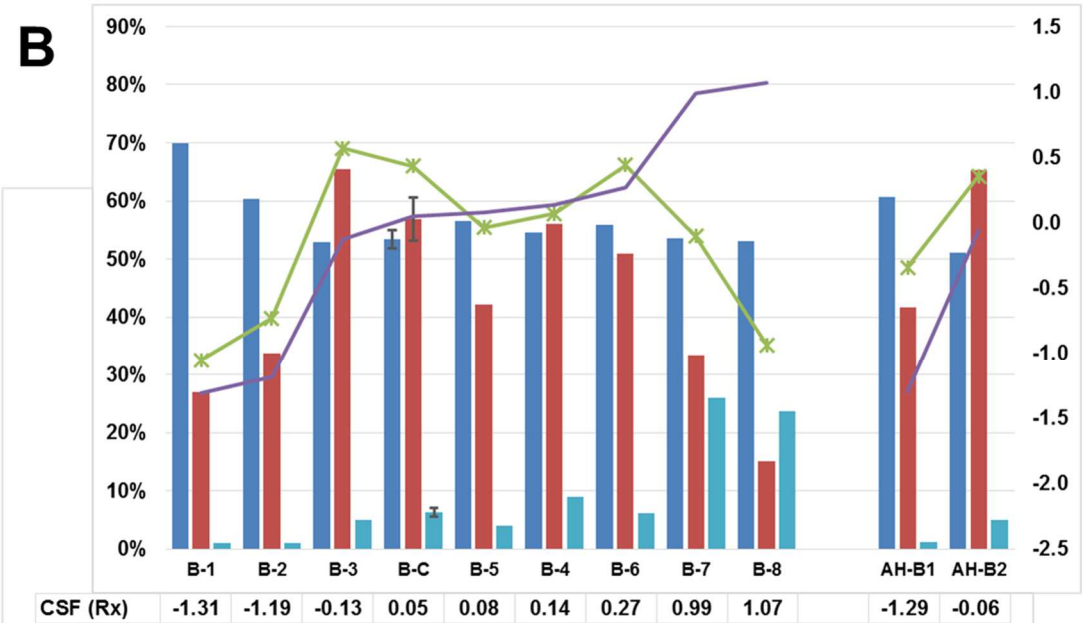
Table 4-5 Comparison of HTT experimental results from different BSG

Feedstock BSG	Hydrothermal treatment (Temp/Time/Acid)	XOS yield (wt.%)	ArOS yield (wt.%)	TXeq yield (wt.%	XOS%[a] (wt.%)	ArOS/ XOS	dgp[b] (g/100g)	ArOS+XOS /TDS[c]	CSF
BSG	**Autocatalytic[d]**								
Carvalheiro et al., (2004)	150°C 20min	38%	n.d.	35.5%	93.4%	n/d	0.71	n.d.	-1.85
(11.1% dm)	170°C 5min	48%	n.d.	47.1%	91.5%	n/d	0.77	n.d.	-1.47
	190°C 5min	61%	n.d.	65.5%	86.5%	n/d	1.89	n.d.	-0.62
Process A (BSG-R)									
(15.3% dm)	**Autocatalytic**								
AH-A1	150°C 10min	41.7%	36.1%	43.1%	96.9%	0.44	0.19	29.7%	-1.71
AH-A2	180°C 5min	65.0%	27.8%	69.9%	93.1%	0.25	1.11	26.7%	-0.89
AH-A3	180°C 15min	78.0%	25.5%	86.4%	90.3%	0.17	1.50	24.1%	-0.49
ELA Run	**ELA catalysed**								
A-C[e]	150°C 10min 12.5mg[e]	61.5%	38.5%	65.3%	94.2%	0.32	0.36	24.3%	-0.56
A-3	170°C 5min 5mg	67.5%	42.6%	71.5%	94.4%	0.32	0.71	25.3%	-0.84
A-7	170°C 5min 20mg	72.6%	22.8%	92.6%	78.5%	0.16	1.31	20.2%	0.28
A-4	170°C 15min 5mg	76.4%	29.7%	83.3%	91.7%	0.20	1.18	25.1%	-0.55
Process B (BSG-SPD)									
(25.0% dm)	**Autocatalytic**								
AH-B1	150°C 10min	41.7%	45.0%	42.8%	97.2%	0.49	0.56	32.5%	-1.29
AH-B2	180°C 5min	65.3%	32.1%	70.2%	93.0%	0.20	1.49	37.8%	0.05
ELA Run	**ELA catalysed**								
B-C[d]	150°C 10min 12.5mg[f]	56.9%	26.8%	63.2%	90.1%	0.21	0.92	28.6%	0.09
B-3	170°C 5min 5mg	65.5%	35.1%	70.3%	93.0%	0.24	1.14	34.9%	-0.13
B-7	170°C 5min 20mg	33.4%	10.1%	59.3%	56.3%	0.14	2.51	14.9%	0.99
B-4	170°C 15min 5mg	56.0%	17.9%	64.8%	86.3%	0.14	1.29	29.5%	0.14
	Acid catalysed and 20 Bar CO_2								
B-10C	150°C 10min 12.5mg	46.7%	8.5%	61.2%	76.2%	0.08	1.44	21.1%	0.15

a XOS percentage of total xylan equivalent recovered
b Degradation products (combined acetic acid, formic acid, furfural and HMF)
c Total dissolved solids in hydrolysate
d Mean of three replicates 12.5mg/g dry feedstock (This study)



CSF (Rx)	-1.81	-1.48	-0.84	-0.56	-0.55	-0.38	-0.36	0.28	0.46	-1.71	-0.30	-0.40
Temp (°C)	130	130	170	150	170	130	130	170	170	150	180	180
Time (min)	5	15	5	10	15	15	5	5	15	10	5	15
Acid (mg/g)	5	5	5	12.5	5	20	20	20	20	0	0	0



CSF (Rx)	-1.31	-1.19	-0.13	0.05	0.08	0.14	0.27	0.99	1.07	-1.29	-0.06
Temp (°C)	130	130	170	150	130	170	130	170	170	150	180
Time (min)	5	15	5	10	5	15	15	5	15	10	5
Acid (mg/g)	5	5	5	12.5	20	5	20	20	20	0	0

Figure 4-3 Comparison between ELA and autocatalytic HTT results: (A) BSG-R and (B) BSG-SPD using combined severity: [■] Insoluble solid residue, [■] XOS yield, [■] Xylose yield, [✳] Hemicellulose yield, [—] CSF (Right axes)

4.3.4 Effect of screw press dewatering on acidification in HTT

The resulting hydrolysate pH from the factorial and autocatalytic pre-screening runs were assessed to investigate the effect of screw pressing dewatering on HTT acidification. In HTT the rate of solubilisation and depolymerisation of xylan and glucan polysaccharides is related proportionally to H+ concentration in the hydrolysate (Mosier, Ladisch and Ladisch, 2002; Negahdar, Delidovich and Palkovits, 2016). Therefore final hydrolysate H+ concentrations were determined and compared to establish possible effects of changes in BSG composition, water content and buffering capacity by the screw press dewatering on acidification in HTT (mol H+ per gram dry BSG).

4.3.4.1 Apparent acidification in autocatalytic LHW HTT proportional to water reduction

In autocatalytic HTT the screw press dewatering of BSG can be consistent with a moisture reduction step. Acidification results from autocatalytic LHW HTT shown in Figure 4-4 for raw BSG-R (5.5 g water/g dm) and screw pressed BSG-SPD (3.0 g water/g dm) resulted comparable apparent acidification (mol H+ produced per gram BSG dry) for each temperature 150 ºC and 180 ºC and the resulting pH and H+ concentrations obtained are equivalent to a constant HTT acidification, mol H+ produced per gram BSG dry, for both 15% and 25% dm BSG in autocatalytic LHW HTT, at temperature 150 ºC (*circa* 2.5 x 10^{-7} mol H+/g dry BSG) and 180 ºC (*circa* 8.4 x 10^{-7} mol H+/g dry BSG). Additional acidifying effects, as a result of the screw press dewatering of BSG-R, resulted in <20% increase in mol H+ released per gram of BSG in autocatalytic LHW HTT in the range of process conditions investigated (Table 4-6). The resulting ratio, R[H+], of mol H+ per gram BSG-SPD, relative to BSG-R, in autocatalytic HTT was 1.16 (±0.18) and 1.09 (±0.06) for 150 °C 10 min (Run AH-A1/B1) and 180 °C 5 min (Run AH-A2/B2) respectively (Table 4-6). Therefore, during autocatalytic LHW HTT in the Parr, the fractionation of selective compounds, including ash, starch and protein, with the screw press dewatering of BSG-R resulting in BSG-SPD did not significantly change the H+ per gram dry BSG released. In autocatalytic LHW HTT, compared to screw press dewatering, temperature is a more significant factor in the BSG hydrolysate acidification. Results show the moisture reduction of 47% between raw (BSG-R) to screw pressed BSG (BSG-SPD) produced only *ca* 100% increased H+ concentration in LHW HTT however a 9% increased temperature (150 ºC to 180 ºC) resulted in a significantly increased, > 230% in H+ concentration (Figure 4-4). Observed differences in the H+ concentrations were proportional to the differences in the water content. However for ELA dosed LHW HTT the screw press dewatering had a much greater increased acidification.

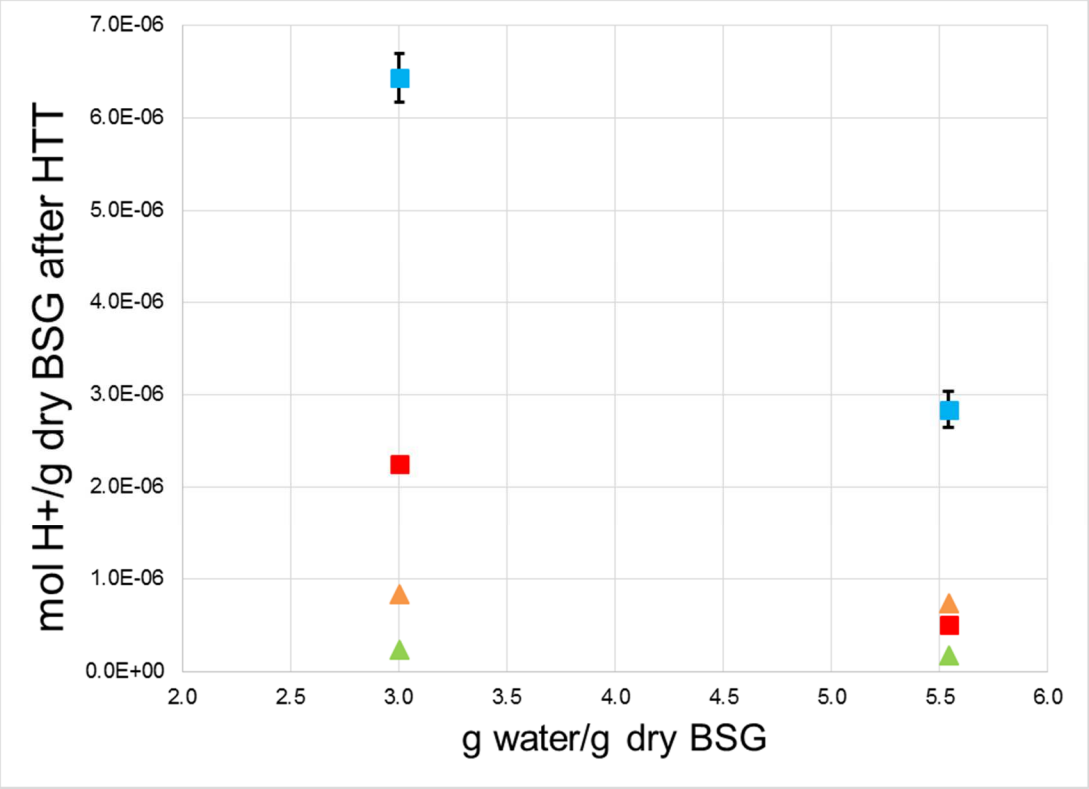


Figure 4-4 The effect of screw press dewatering on HTT acidification: Autocatalytic LHW at [▲] 150 ºC for 10 min and [▲] 180 ºC for 5 min, and ELA dosed LHW at [■] 150 ºC for 10 min with 12.5 mg H_2SO_4/g dm and [■] 170 ºC for 5 min with 5 mg H_2SO_4/g dm.

4.3.4.2 Screw press dewatering improved acidification in ELA dosed LHW HTT

The screw press dewatering of BSG resulted in significant additional acidifying effects in ELA catalysed LHW HTT. Additional acidifying effects in ELA dosed LHW HTT, as a result of the screw press dewatering of BSG-R, increased up to 400% the mol H+ released per dry gram of BSG in BSG-SPD with ELA dosing of only 5 mg H_2SO_4/g dm at 170 ºC for 5 min (Figure 4-4). The resulting ratio, R[H+], or mol H+ per gram BSG-SPD relative to BSG-R, after the ELA HTT treatment over the range of process conditions investigated was found between 1.08 to 4.01 (Table 4-6). Results from ANOVA of effects on R[H+] show indeed the effect of temperature and interactions were significant, with a p-value below 0.05 (Figure 4-5 A).Both the treatment time and its interaction with temperature were found just about significant (Figure 4-5 A). As shown by Figure 4-5 B, the increased treatment time in ELA dosed LHW HTT, especially at high temperatures (170 °C), lead to a decreased acidifying effect with screw pressing; while at lower temperatures (130 °C), the opposite effect was found. The increased acidification in ELA dosed LHW HTT of BSG-SPD as a result of screw press dewatering lead to increased H+ concentration (up to ca 640%) in the resulting hydrolysates significantly more than expected by ca 47% moisture reduction from the screw press dewatering of BSG from 15% to 25% dm (Figure 4-4). The increased H+ generated with BSG-SPD in ELA dosed LHW HTT can be a combined effect of reduced water content, reduced buffering capacity and

compositional changes caused by screw press dewatering. The increased effect of ELA acid dosing on XOS yield from BSG as a result of the screw press dewatering is confirmed by significant curvature found in statistical analysis of XOS yields from BSG-SPD (Appendix Figure 4A-1). An additional quadratic term introduced for acid loading could account for the increased effect of acid loading (Appendix Figure 4A-2 B).

As shown above, especially with ELA dosing, screw press dewatering can improve the hydrolysate acidification, yet the solid BSG contains a large part of the buffering capacity of BSG that can neutralise HTT acidification. The resulting H+ concentration showed a negative relationship with ELA HTT treatment time as the hydrolysate pH increased for both BSG raw and screw pressed (Table 4-6) at temperatures of 130 ºC in ELA HTT at 5 min (run A5/B-5 with 20 mg H_2SO_4/g dm) the pH obtained (pH 2.40/1.84) increase (pH 2.89/1.95) at 15 min.

Table 4-6 Comparison between resulting hydrolysate pH from BSG-R (15% dm) and BSG-SPD (25% dm) obtained by autocatalytic LHW and ELA dosed HTT

	Autocatalytic		ELA dosed HTT								
Run nr	**AH1**	**AH2**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10avg[a]**
Temperature (°C)	**150**	**180**	**130**	**130**	**170**	**170**	**130**	**130**	**170**	**170**	**150**
Time (min)	**10**	**5**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**10**
Acid (mg/g dm)	**0**	**0**	**5**	**5**	**5**	**5**	**20**	**20**	**20**	**20**	**12.5**
BSG-R (15% dm)	**Run AH-A1/2**		**ELA Run A-1 to A-11**								
CSF	-1.71	-0.30	-1.81	-1.48	-0.84	-0.55	-0.36	-0.38	0.28	0.46	-0.56
pH	4.38	3.84	3.82	3.80	3.99	3.89	2.40	2.59	2.88	2.90	3.28
mol H+ ELA /start [b]	-	-	1.5	1.5	1.5	1.5	5.9	5.9	5.9	5.9	3.7
%mol H+ ELA of final	-	-	12.2%	11.6%	18.0%	14.3%	1.9%	2.9%	5.6%	5.9%	8.8%
BSG-SPD (25% dm)	**Run AH-B1/2**		**ELA Run B-1 to B-11**								
CSF	-1.29	-0.06	-1.31	-1.19	-0.13	0.14	0.08	0.27	0.99	1.07	0.05
pH	4.05	3.54	3.37	3.50	3.12	3.20	1.84	1.95	2.12	2.30	2.67
mol H+ ELA /start [b]	-	-	2.7	2.7	2.7	2.7	10.8	10.8	10.8	10.8	6.7
%mol H+ ELA of final	-	-	8.0%	10.7%	4.5%	5.4%	1.0%	1.2%	1.8%	2.7%	3.9%
R[H+] Resulting ratio[c] mol H+ (25%) / mol H+ (15%)	**1.16**	**1.09**	**1.53**	**1.08**	**4.01**	**2.65**	**1.95**	**2.35**	**3.10**	**2.16**	**2.23**

[a] Average of three runs; [b] Starting pH = 4.9; [c] R[H+] (mol H+/ g dry BSG)

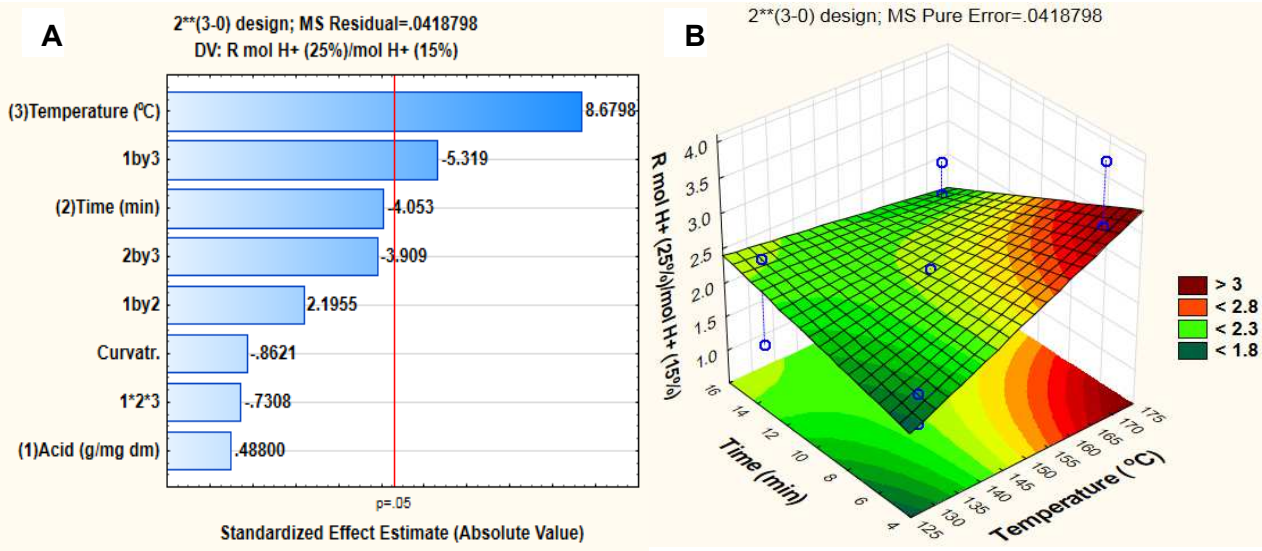


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Figure 4-5 Resulting ratio of mol H+ /g dry BSG obtained for BSG-SPD relative to BSG-R (g dry BSG basis) in the hydrolysate after ELA HTT (A) Standardized Pareto chart of effects (B) Surface plot of effect of time and temperature with 20 mg H_2SO_4/ g dry BSG

4.3.4.3 Screw press dewatering and chemical compositions in HTT

The changes in chemical composition between BSG-R and BSG-SPD with screw press dewatering did not affect, the main HTT mechanisms taking place in solubilisation and depolymerisation of BSG hemicellulose (Figure 4-6). No significant difference in monomeric sugars formation relative to oligomers for GlcOS and XOS can be seen when comparing products in hydrolysates from BSG-R and BSG-SPD after ELA catalysed HTT (Figure 4-6). ELA loadings and screw press dewatering had a significant effect on the extent of depolymerisation of the glucan and xylan in the HTT, while the depolymerisation follow proportionally for both glucan and xylan. However, contribution of the screw press dewatering effect to the changes in H+ concentration is less when compared to the ELA loading. Under the same process conditions, the relative monomeric and oligomeric yields were identical for autocatalytic runs for BSG-R and BSG-SPD. However, with equal H_2SO_4 ELA dosing to both BSG-R and BSG-SPD, the interaction between acid loading and temperature resulted in an increased extent of polysaccharide depolymerisation to monomeric sugars xylose and glucose with the BSG-SPD.

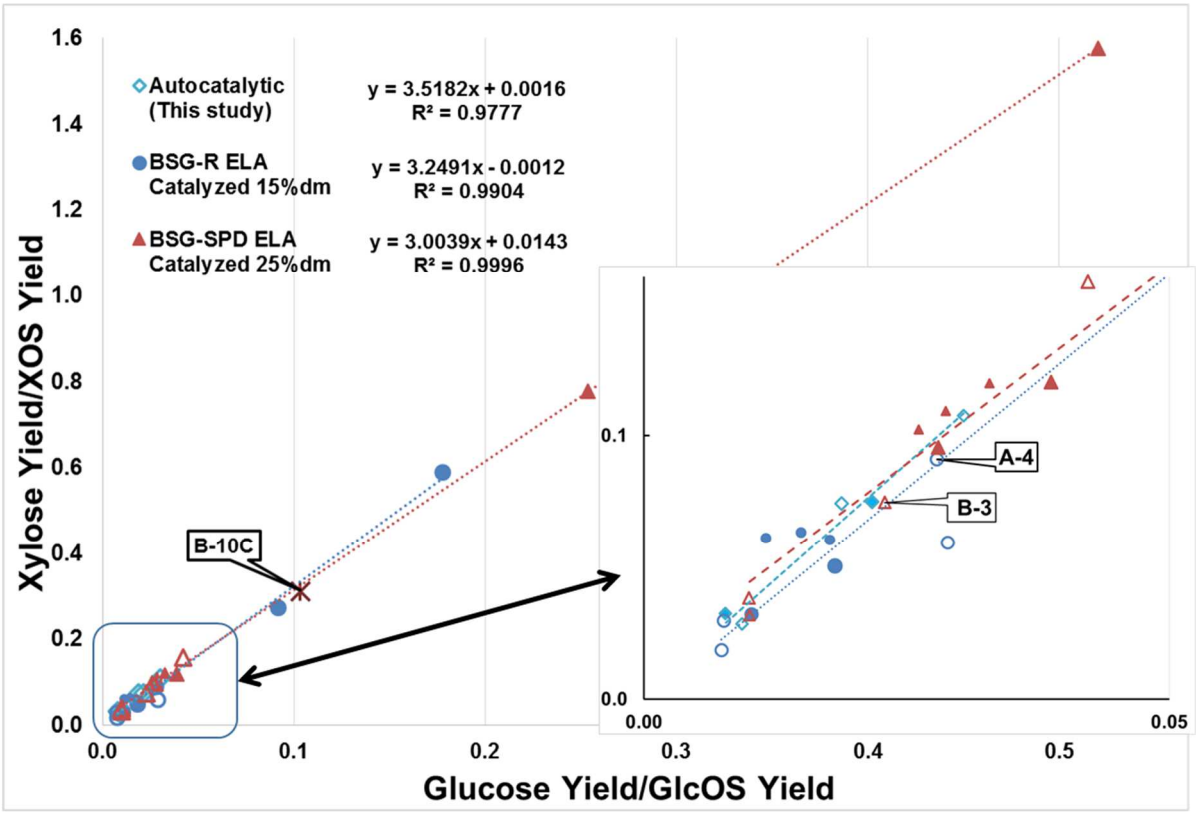


Figure 4-6 Comparison of HTT yields of oligomeric and reducing sugars: autocatalytic [◇] BSG-R 15% dm and [◆] BSG-SPD 25% dm and ELA for BSG-R [○] 5 mg and [●] 20 mg H_2SO_4/g dm and ELA for BSG-SPD [△] 5 mg and [▲] 20 mg H_2SO_4 /g dm (enlargement insert for autocatalytic HTT range).

4.3.4.4 Extending solubilisation and depolymerisation by addition of CO_2

As a "Green" alternative catalyst, CO_2 used with ELA HTT showed increased effective depolymerisation and reduction of XOS to xylose monomeric sugar (Figure 4-6). The preliminary test, using BSG-SPD at centre runs of 150 °C and 10 min with additional 20 Bar CO_2 (run B-10C), showed that added CO_2 resulted in a reduced pH in the hydrolysate, from pH 2.67 (with no CO_2) to pH 2.61. The CO_2 effectively increased the [H+} concentration in the hydrolysate by 15%. The XOS fraction in the xylan recovered decreased from 90.1% to 76.2% while the total xylan recovered yield reduced only marginally from 63.2% to 61.2% (Table 4.5). The preliminary test run B-10C, had a significant increase in the degradation products formed from 0.92 to 1.44 g/100 g dry BSG while the CSF increased from 0.09 to 0.15 compared to run B-10 without CO_2 (Table 4.5). Although literature reports the use of CO_2 addition in autocatalytic HTT to aid the autocatalytic HTT process (Gurgel, Pimenta and Curvelo, 2014; Morais, Mata and Bogel-Lukasik, 2014; Luft *et al.*, 2018), this novelty shows that CO_2 can manipulate the severity in combination with H_2SO_4 at moderate pressures. Even lower CO_2 pressures can be investigated for control on hemicellulose solubilisation and XOS yield.

4.3.5 Xylobiose and xylotriose yield in XOS

The distribution of degree of polymerisation of the XOS produced in ELA HTT process varies with the ELA HTT process conditions. The short chain xylan oligomers, xylobiose (X2) and xylotriose (X3) are of special interest for their prebiotic effect. They can be produced (de Moura, Macagnan and da Silva, 2015; Gomez *et al.*, 2015), and maximised in the XOS fraction. This would minimise or avoid the need for a subsequent post HTT step for the production of the X2 and X3. With ELA catalysed HTT, the highest combined fractions of X2 and X3 in the XOS were 19.6% and 23.0 for BSG-R and BSG-SPD respectively. Both were obtained at 170 °C with 20 mg H_2SO_4/g dm acid loading (Figure 4-6). Both of these results are higher than the 16.7% reported by Carvalheiro et al., (2004b), obtained at 190 °C through autocatalytic HTT at their process conditions for maximum XOS yield. For both BSG-R and BSG-SPD, the effect of a rising combined severity factor (CSF) showed increased yield of X2 and X3 in the XOS product (Figure 4-6). Therefore, adjusting the CSF with ELA loading, can increase the X2 and X3 fractions in XOS, in a single step process. For BSG-R, the XOS produced at the highest XOS yield (run A-4) contained 11.2% of X2 and X3 combined. However, an increase in acid loadings from 5 mg to 20 mg H_2SO_4 /g dm at the same process conditions (run A-7) led to a near doubling of the yield (19.6%). Similarly, using BSG-SPD (run B-7), the combined X2 and X3 yield in XOS increased from 10.8% to 23.0%, but at a much lower XOS yield and total xylan equivalent yield.

The preliminary HTT screening of BSG-R and BSG-SPD with more than 40 mg H_2SO_4/g dm acid loadings, increased the combined X2 and X3 yields to more than 30% of the XOS fraction while the total xylan equivalent yield was above 80% (Figure 4-6 run A-Z and B-Z). Incorporating a CO_2 catalyst significantly increased the X2 and X3 yield in the XOS produced. In run B-C at 150 °C with BSG-SPD, the addition of 20 Bar CO_2 (run B-10C), resulted in a near doubling of the X2 and X3 yield in XOS from 7.2% to 15.6%, while the resulting total xylan equivalent yield was maintained (Figure 4-6). Thus, the further use of ELA acid dosage with CO_2 could be investigated as a tool for XOS production to optimise short chain oligomers including X2 and X3. Ideally, as “Green Chemistry”, CO_2 can be used alone and replace additional acid usage in XOS production.

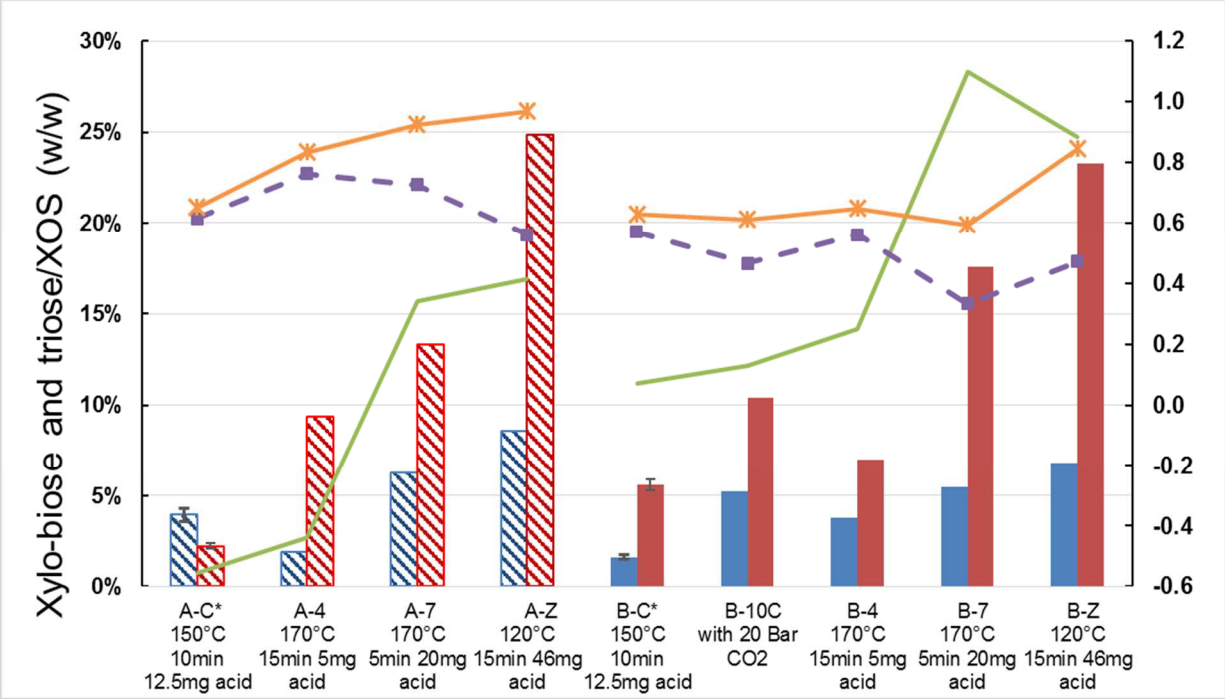


Figure 4-7 XOS composition: [/] Xylobiose (X2) and [/] xylotriose (X3) from ELA HTT (Left axis; Yield in XOS, Dashed [//] for BSG-R; Solid [/] for BSG-SPD, Right axis; [] CSF, [] XOS Yield, and [] Xylan Eq Yield)(* average for values for triplicate runs used).

4.3.6 Effect of screw press dewatering on the resulting hydrolysate from HTT

BSG is heterogeneous and fractionation of BSG hemicellulose in HTT inevitably generates interactions between components that negatively affects the yields and purity of the XOS fraction. Hence, another important factor to improve on in HTT is the fraction of XOS and ArOS in the total dissolved solids (TDS) of the hydrolysate. Protein reduction in BSG for HTT is key since proteinaceous compounds make up the majority of the non-volatile components in the hydrolysate. Protein contributes to the buffering effect in HTT and can react with oligomers and sugars which can reduce their yields. The screw press dewatering of BSG-R had a significant effect on the composition of solubles in the hydrolysate.

4.3.6.1 XOS concentration and TDS from BSG-R and BSG-SPD

The 50% reduction in moisture content through dewatering BSG-R with a screw press had a significant positive effect on the XOS concentration obtained by ELA HTT and increased the purity of the XOS. A XOS concentration of 31.7 g/l was obtained form BSG-SPD with maximum XOS yield (run B-3), almost double the 16.6 g/l obtained from raw BSG-R. The highest xylose concentrations obtained for BSG-R was 7.2 g/l and 14.7 g/l for BSG-SPD both at 170 °C and 20 mg H_2SO_4 /g dm acid dosing for 15 min and 5 min respectively (Appendix

Table 4A-2 and Table 4A-3). Additionally, the hydrolysate of BSG-SPD (run B-4) contained a greater fraction (34.9%) of oligomeric products - XOS and ArOS - in the TDS of the hydrolysate, compared to the 25.1% (run A-4) obtained from raw BSG-R (Table 4-5). These increased XOS and ArOS concentrations in the TDS of the BSG-SPD hydrolysate was achieved through the increased fibre content by means of screw press dewatering. The screw press dewatering step selectively reduced the ash, GlcOS and proteinaceous compounds, as soluble and insoluble solids. Since it is reported that up to 80% of XOS production cost is due to purification cost, applying a screw press processing step before ELA HTT can decrease overall XOS production costs with the increased XOS concentration and fraction of XOS in TDS of resulting hydrolysate (Amorim, Silvério and Rodrigues, 2019).

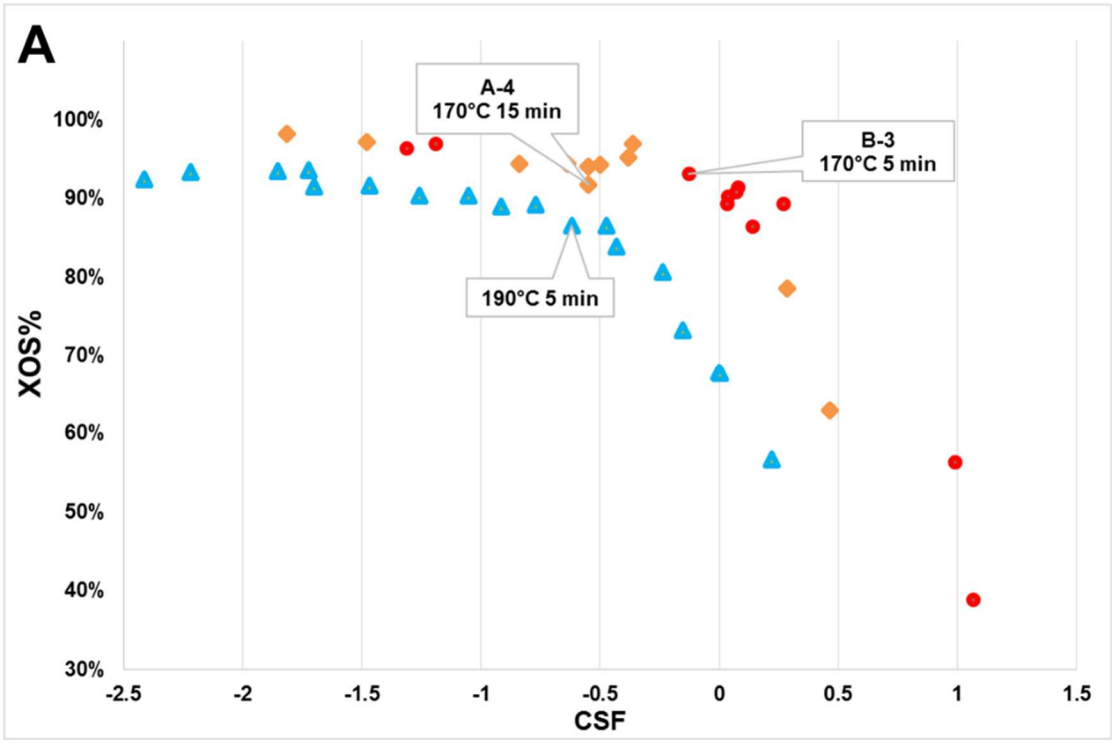
4.3.6.2 Protein from screw press dewatering BSG-R

Separation of a high protein fraction with the screw press before HTT is more advantageous since it provides higher protein recovery, and less solubilisation in the hydrolysate. Proteinaceous compounds in the BSG-SPD were significantly reduced by screw pressing prior to HTT and produced a valuable, protein rich (43 wt.% total amino acid content) insoluble solid fraction in the press liquid with potential for protein extraction as a co-product to XOS in a multiproduct biorefinery scenario (Appendix Table 4A-7). The insoluble solid fraction, recovered from the press liquid in this study, contained a lower protein content than previously reported values (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977). This lower protein yield can be as a result of the light pressing applied. Despite the low protein yield in the pressed liquid, the reduction in protein content in BSG-SPD is reflected in the ELA HTT results as the other non-determined dissolved solubles (NDS), including the proteinaceous compounds in the hydrolysate, decreased from 13.2 g/l for BSG-R (run A-4), to 9.0 g/l for BSG-SPD (run B-3), both at their highest XOS yields (Appendix Table 4A-2 and Table 4A-3). These lower NDS values are in agreement with the crude protein recovery achieved using the screw pressed BSG in HTT. Only 45.4% of the crude protein remains in the insoluble solid after ELA HTT of BSG-R at the conditions for the highest XOS yield (run A-4), compared to the 70.9% protein recovered from BSG-SPD (run B-3), when including the crude protein in the screw press liquid (solid compositions given in Appendix Figure 4A -6). On the other hand, the ELA HTT can be considered as an enrichment process since the amino acid profiles of the remaining solids showed, for both BSG's, that the basic amino acids were hydrolysed preferentially concentrating essential amino acids in the remaining solids (Appendix Table 4A -7). Therefore, the separation of a high protein fraction with the screw press together with the residual protein obtained from ELA HTT can be advantageous in a valorisation case for BSG.

4.3.7 CSF effect on xylan recovery and XOS yield

ELA dosing in LHW HTT increased accumulation of XOS in the recovered xylan equivalent (XOS%) compared to autocatalytic LHW HTT. ELA HTT resulted in more accumulation of XOS in the recovered xylan equivalent (XOS%) of 91.7% (BSG-R run A-4 at 170 ºC) and 93.0% (BSG-SPD run B-3 at 170 ºC), compared to reported autocatalytic LHW HTT results of 86.5% (190 ºC and CSF =-0.62) as shown in Figure 4-8 A. The higher XOS% were obtained at higher CSF's (-0.55 and -0.13) as a result of the ELA addition. This XOS accumulation in the hydrolysate from ELA HTT occurred due to the increased H+ concentration that led to an increased rate coefficient for xylan solubilisation to XOS (Mosier, Ladisch and Ladisch, 2002), compensating for the negative effect of lower temperatures (170 ºC compared to 190 ºC). At the reduced temperatures, XOS depolymerisation to xylose and also furfural formation was reduced (Shen and Wyman, 2011).

ELA dosed HTT of BSG-R (15% dm) resulted in a higher xylan equivalent yield of >85% compared to the reported autocatalytic HTT data (11% dm) for BSG (xylan equivalent yield of <66%), as shown in Figure 4-8 B, which is described as a direct result of the acid catalyst (Shen and Wyman, 2011). However, mass and heat transfer limitations inherent with high solids loadings from ELA HTT of BSG-SPD in the Parr system, resulted in a drop in xylan equivalent yield at higher CSF (<70%) compared to the more dilute and homogeneous slurry of BSG-R. A comparison with a pilot scale ELA HTT of rice straw conducted at >24% dry matter content in a high solids loading screw reactor (Kapoor *et al.*, 2017), show high xylan recovery (>78%) can be obtained at increased CSFs when using high solids loading processing equipment (Figure 4-8 B). This suggests that for BSG-SPD, higher xylan recovery and increased XOS yields can be targeted in similar screw type reactor vessels or steam explosion reactors that are more suitable for high solids loading HTT's. This highlights the applicability of ELA dosing and the use of bench scale BSG ELA HTT data for scale up applications (Lischeske *et al.*, 2016).



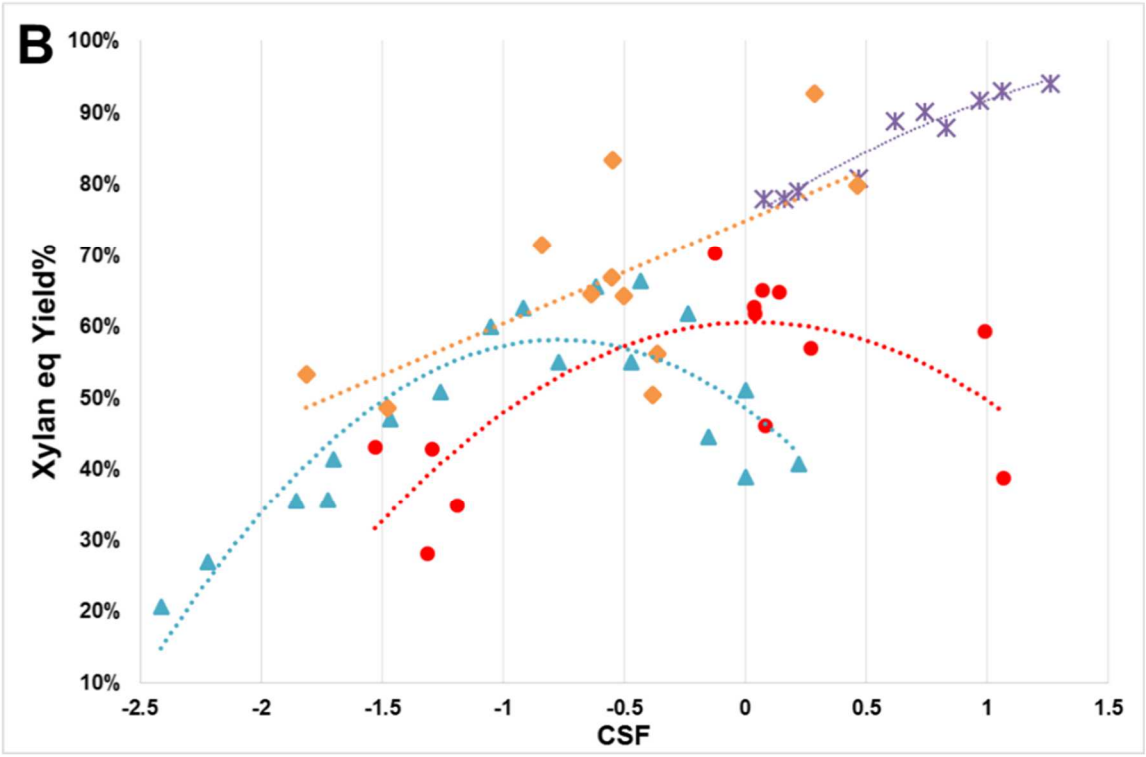


Figure 4-8 Comparison of ELA- and autocatalytic HTT (A) XOS% yield (highest XOS yields of each are indicated) and (B) xylan recovery using CSF ([▲] 11% dm autocatalytic HTT (Carvalheiro *et al.*, 2004), [◆] 15% dm BSG-R ELA (this study), [●] 25% dm BSG-SPD ELA (this study), [✱] 28% dm Rice straw ELA (Kapoor et al 2017)). The lines only show trends.

4.4 Conclusions

Lack of efficient processing technologies of BSG hinders the large-scale valorisation of BSG. XOS produced from BSG have high value applications in novel food, beverage and health product formulations. This study demonstrated a HTT process intensification for production of XOS from raw (15% dm) and screw pressed (25% dm) BSG using ELA dosing in LHW HTT. With the use of ELA dosing, the increased production of degradation products as a result of increased solids loading could be circumvented. Similar and even higher XOS yields (61.4-76.4%), were obtained compared to reported autocatalytic LHW HTT process (9-11% dm) yields (61% at 190 ºC), and even at lower temperature requirements (150-170 °C) together with a reduction in degradation products. The screw press dewatering process demonstrated effective moisture reduction in the BSG for process intensification in biorefining. Additional to the moisture content reduction, the screw press dewatering step also increased XOS purity in the hydrolysate from 25.3 to 34.1% by increasing the fibre fraction in the BSG. This increase was due to the selective separation of dissolved solids, ash, starch and protein. Screw press dewatering of BSG enhanced the autocatalytic LHW HTT by increasing H+ concentrations and reduced hydrolysate pH's that were found to approximate the reduction in water. On the other hand the ELA dosing further enhanced acidifying effects in ELA dosed LHW HTT with up to 400% increased mol H+ released per gram of BSG in the range investigated. These process improvements demonstrated a significant process intensification in HTT technology that can support BSG valorisation concepts for breweries around the world.

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E-supplementary data of this work can be found in online version of the paper.

4.6 References

Abu-Ghannam, N., Balboa, E., 2018. 9 – Biotechnological, food, and health care applications, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier. https://doi.org/10.1016/B978-0-08-102162-0.00009-5

Amorim, C., Silvério, S.C., Rodrigues, L.R., 2019. One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. Food Chem. 270, 86–94. https://doi.org/10.1016/j.foodchem.2018.07.080

Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam explosion pretreatment of wood: Effect of Chip Size, Acid, Moisture Content and Pressure Drop. Biotechnol. Bioeng. 28, 792–801. https://doi.org/10.1002/bit.260280604

Cardenas-toro, F.P., Alcazar-alay, S.C., Forster-carneiro, T., Meireles, M.A. a, 2014. Obtaining Oligo- and Monosaccharides from Agroindustrial and Agricultural Residues Using Hydrothermal Treatments. Food Public Heal. 4, 123–139. https://doi.org/10.5923/j.fph.20140403.08

Carvalheiro, F., Esteves, M.P., Parajo, J.C., Pereira, H., Gıırio, F.M., 2004. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91, 93–100. https://doi.org/10.1016/S0960-8524(03)00148-2

Carvalheiro, F., Garrote, G., Parajo, J.C., Pereira, H., Gı, F.M., 2005. Kinetic Modeling of Brewery' s Spent Grain Autohydrolysis. Biotechnol.Prog. 21, 233–243.

Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst effects and the combined severity parameter. Appl. Biochem. Biotechnol. 24–25, 1–14. https://doi.org/10.1007/BF02920229

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of Initial Moisture Content and Chip Size on the Bioconversion Efficiency of Softwood Lignocellulosics. Biotechnol. Bioeng. 85, 413–421. https://doi.org/10.1002/bit.10905

de Moura, F.A., Macagnan, F.T., da Silva, L.P., 2015. Oligosaccharide production by hydrolysis of polysaccharides: A review. Int. J. Food Sci. Technol. 50, 275–281. https://doi.org/10.1111/ijfs.12681

Duarte, L.C., Carvalheiro, F., Lopes, S., Marques, S., Pajaró, J.C., Gírio, F.., 2004. Comparison of Two Posthydrolysis Processes of Brewery's Spent Grain Autohydrolysis Liquor to Produce a Pentose-Containing Culture Medium †. Appl. Biochem. Biotechnol. 113–116, 1041–1058.

Finley, J.W., Walker, C.E., Hautala, E., 1976. Utilization of Press Water from Brewers Spent Grains. J. Sci. Food Agric. 27, 655–660.

Galbe, M., Zacchi, G., 2012. Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy 46, 70–78. https://doi.org/10.1016/j.biombioe.2012.03.026

García-Aparicio, M., Parawira, W., Van Rensburg, E., Diedericks, D., Galbe, M., Rosslander, C., Zacchi, G., Görgens, J., 2011. Evaluation of steam-treated giant bamboo for production of fermentable sugars. Biotechnol. Prog. 27, 641–649. https://doi.org/10.1002/btpr.580

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

Gullón, P., González-Muñoz, M.J., Parajó, J.C., 2011. Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. Bioresour. Technol. 102, 6112–6119. https://doi.org/10.1016/j.biortech.2011.02.059

Gurgel, L.V.A., Marabezi, K., Zanbom, M.D., Curvelo, A.A.D.S., 2012. Dilute acid hydrolysis of sugar cane bagasse at high temperatures: A Kinetic study of cellulose saccharification and glucose decomposition. Part I: Sulfuric acid as the catalyst. Ind. Eng. Chem. Res. 51, 1173–1185. https://doi.org/10.1021/ie2025739

Gurgel, L.V.A., Pimenta, M.T.B., Curvelo, A.A. da S., 2014. Enhancing liquid hot water (LHW) pretreatment of sugarcane bagasse by high pressure carbon dioxide (HP-CO2). Ind. Crops Prod. 57, 141–149. https://doi.org/10.1016/j.indcrop.2014.03.034

Huige, N.J., 1994. Handbook of Brewing: In Food Science and Technology, in: Hardwick, W.A. (Ed.), Marcel Dekker Inc. CRC Press, pp. 501–550). https://doi.org/10.1002/9780470290118

Jay, A.J., Parker, M.L., Faulks, R., Husband, F., Wilde, P., Smith, A.C., Faulds, C.B., Waldron, K.W., 2008. A systematic micro-dissection of brewers' spent grain. J. Cereal Sci. 47, 357–364. https://doi.org/10.1016/j.jcs.2007.05.006

Jönsson, L.J., Martín, C., 2016. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour. Technol. 199, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Kapoor, M., Soam, S., Agrawal, R., Gupta, R.P., Tuli, D.K., Kumar, R., 2017. Pilot scale dilute acid pretreatment of rice straw and fermentable sugar recovery at high solid loadings. Bioresour. Technol. 224, 688–693. https://doi.org/10.1016/j.biortech.2016.11.032

Kerby, C., Vriesekoop, F., 2017. An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3, 1–12. https://doi.org/10.3390/beverages3020024

Leibbrandt, N.H., Knoetze, J.H., Görgens, J.F., 2011. Comparing biological and thermochemical processing of sugarcane bagasse: An energy balance perspective. Biomass and Bioenergy 35, 2117–2126. https://doi.org/10.1016/j.biombioe.2011.02.017

Liao, W., Liu, Y., Liu, C., Chen, S., 2004. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material - Dairy manure. Bioresour. Technol. 94, 33–41. https://doi.org/10.1016/j.biortech.2003.11.011

Lischeske, J.J., Crawford, N.C., Kuhn, E., Nagle, N.J., Schell, D.J., Tucker, M.P., McMillan, J.D., Wolfrum, E.J., 2016. Assessing pretreatment reactor scaling through empirical analysis. Biotechnol. Biofuels 9, 213. https://doi.org/10.1186/s13068-016-0620-0

López-Linares, J.C., García-Cubero, M.T., Lucas, S., González-Benito, G., Coca, M., 2019. Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production. Chem. Eng. J. 368, 1045–1055. https://doi.org/10.1016/j.cej.2019.03.032

Luft, L., Confortin, T.C., Todero, I., Ugalde, G., Zabot, G.L., Mazutti, M.A., 2018. Transformation of residual starch from brewer's spent grain into fermentable sugars using supercritical technology. J. Supercrit. Fluids 140, 85–90. https://doi.org/10.1016/j.supflu.2018.06.006

Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. J. Inst. Brew. 4, 553–568. https://doi.org/10.1002/jib.363

Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment-A review. Biotechnol. Bioeng. 109, 1430–1442. https://doi.org/10.1002/bit.24464

Morais, A.R.C., Mata, A.C., Bogel-Lukasik, R., 2014. Integrated conversion of agroindustrial residue with high pressure CO2 within the biorefinery concept. Green Chem. 16, 4312–4322. https://doi.org/10.1039/C4GC01093K

Mosier, N.S., Ladisch, C.M., Ladisch, M.R., 2002. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. 79, 610–618. https://doi.org/10.1002/bit.10316

Moura, P., Carvalheiro, F., Esteves, M.P., Gírio, F.M., 2008. Prebiotic xylo-oligosaccharides as high-value co-products on an integrated biorefinery approach from lignocellulosic feedstock, in: International Conference and Exhibition on Bioenergy April 6th – 9th 2008 Universidade Do Minho, Guimarães, Portugal.

Mussatto, S.I., Dragone, G., Roberto, I.C., 2006. Brewers' spent grain: Generation, characteristics and potential applications. J. Cereal Sci. 43, 1–14. https://doi.org/10.1016/j.jcs.2005.06.001

Mussatto, S.I., Roberto, I.C., 2005. Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J. Sci. Food Agric. 85, 2453–2460. https://doi.org/10.1002/jsfa.2276

Negahdar, L., Delidovich, I., Palkovits, R., 2016. Aqueous-phase hydrolysis of cellulose and hemicelluloses over molecular acidic catalysts: Insights into the kinetics and reaction mechanism. Appl. Catal. B Environ. 184, 285–298.

Robertson, J.A., I'Anson, K.J.A., Brocklehurst, T.F., Faulds, C.B., Waldron, K.W., 2010. Effect of storage conditions on the microbial ecology and biochemical stability of cell wall components in brewers spent grain. J. Agric. Food Chem. 58, 7266–7272. https://doi.org/10.1021/jf1001099

Rommi, K., Niemi, P., Kemppainen, K., Kruus, K., 2018. Impact of thermochemical pre-treatment and carbohydrate and protein hydrolyzing enzyme treatment on fractionation of protein and lignin from brewer's spent grain. J. Cereal Sci. 79, 168–173. https://doi.org/10.1016/j.jcs.2017.10.005

Rosa, M., Beloborodko, A., 2015. A decision support method for development of industrial synergies : case studies of Latvian brewery and wood-processing industries. J. Clean. Prod. 105, 461–470. https://doi.org/10.1016/j.jclepro.2014.09.061

Shen, J., Wyman, C.E., 2011. A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. Bioresour. Technol. 102, 9111–9120. https://doi.org/10.1016/j.biortech.2011.04.001

Skendi, A., Harasym, J., Galanakis, C.M., 2018. 7 – Recovery of high added-value compounds from brewing and distillate processing by-products, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier Science. https://doi.org/10.1016/B978-0-08-102162-0.00007-1

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J. Agric. Food Chem. 58, 9043–9053. https://doi.org/10.1021/jf1008023

Steiner, J., 2016. Brewer's spent grain—Waste material as potential raw material for a healthy diet, in: World Brewing Congress, Denver Colorado, USA, August 13-17, 2016. pp. 2–3.

Stiles, R.S., Herbert, H.S., 1977. Centrifugal Separation, Isolation and Characteristics of Brewery Spent Grains and Press Liquor. MBAA Tech. Q. 14, 21–34.

Sui, W., Chen, H., 2015. Study on loading coefficient in steam explosion process of corn stalk. Bioresour. Technol. 179, 534–542. https://doi.org/10.1016/j.biortech.2014.12.045

Thomas, K.R., Rahman, P.K.S.M., 2006. Brewery wastes. Strategies for sustainability. A review. Asp. Appl. Biol. 80, 147–153.

Vallejos, M.E., Zambon, M.D., Area, M.C., Curvelo, A.A. da S., 2012. Low liquid–solid ratio (LSR) hot water pretreatment of sugarcane bagasse. Green Chem. 14, 1982. https://doi.org/10.1039/c2gc35397k

Vegas, R., Alonso, L., Domıniques, H., Parajó, J.C., 2005. Manufacture and Refining of Oligosaccharides from Industrial Solid Wastes. Ind. Eng. Chem. Res 44, 614–620.

Wang, B., Luo, Y., Myung, K.H., Liu, J.X., 2014. Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grains. Asian-Australasian J. Anim. Sci. 27, 832–840. https://doi.org/10.5713/ajas.2013.13668

Weber, B., Stadlbauer, E.A., 2017. Sustainable paths for managing solid and liquid waste from distilleries and breweries. J. Clean. Prod. 149, 38–48. https://doi.org/10.1016/j.jclepro.2017.02.054

Weger, A., Binder, S., Franke, M., Hornung, A., 2014. Solid Biofuel Production by Mechanical Pre-Treatment of Brewers' Spent Grain. Chem. Eng. Trans. 37, 661–666. https://doi.org/10.3303/CET1437111

Weger, A., Jung, R., Stenzel, F., Hornung, A., 2017. Optimized Energetic Usage of Brewers' Spent Grains. Chem. Eng. Technol. 40, 306–312. https://doi.org/10.1002/ceat.201600186

Yan, Q., Miazek, K., Grande, P.M., Domínguez de María, P., Leitner, W., Modigell, M., 2014. Mechanical pretreatment in a screw press affecting chemical pulping of lignocellulosic biomass. Energy & Fuels 28, 6981–6987. https://doi.org/10.1021/ef501706w

Yang, B., Tucker, M., 2013. Laboratory Pretreatment Systems to Understand Biomass Deconstruction, in: E., C., Wyman (Eds.), Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals. John Wiley & Sons Inc., pp. 489–521. https://doi.org/10.1002/9780470975831.ch23

Yang, B., Wyman, C.E., 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioprod. Biorefining 2, 26–40. https://doi.org/10.1002/bbb.49.

Appendix: Chapter 4 - ELA LHW HTT experimental data

Table 4A-1 Comparing autohydrolysis with mineral acid catalysed XOS production for BSG with 15% solids loading using 12.5 mg acid/g dm at 150 °C for 10 min.

Acid Catalyst	Acid loading (mol H+/g dm)	XOS yield (wt%)	XOS%[a] (wt.%)	TDS yield[b] (wt.%)	TRS[c] (g/100g)	CSF	Cost Acid $/kg dm
None	0	41.7%	96.9%	26.8%	1.77	-1.71	$0.00
H_2SO_4[d]	0.26	62.2%	94.2%	38.4%	3.85	-0.56	$1.51
HCl	0.34	69.6%	88.0%	51.3%	6.49	-0.27	$5.75
H_3PO_4	0.38	56.8%	96.6%	35.8%	2.73	-1.15	$2.17
HNO_3	0.20	71.7%	94.7%	42.0%	4.03	-0.87	$4.96

[a] XOS percentage of total xylan equivalent recovered
[b] Total dissolved solids hydrolysate
[c] Total reducing sugars per 100 gram dry feedstock
[d] Mean of three replicates (This study)

ELA HTT results from full factorials with centre points, Table 4A-2 for BSG-R (Run A1-A11) and Table 4A-3 for BSG-SPD (Run B1-B11).

Table 4A-2 Results for acid catalysed XOS production for BSG-R

Run	Independent variables[a]			XOS (g/l)	XOS yield (wt%)	ArOS yield (wt%)	XOS%[b] (wt.%)	ArOS%[c] (wt.%)	Inhibitors[d] (g/100g)	TDS[e] yield (wt.%)	TDS ND[f] (g/100g)	Mass balance closure	CSF
	AC	t	T										
A-1	5	5	130	11.39	52.4%	66.5%	98.2%	90.8%	0.10	39.8%	16.51	95.6%	-1.81
A-2	5	15	130	10.89	47.2%	45.9%	97.1%	74.4%	0.15	32.6%	5.95	91.2%	-1.48
A-3	5	5	170	14.54	67.5%	42.6%	94.4%	56.1%	0.71	41.2%	10.94	89.9%	-0.84
A-4	5	15	170	16.57	76.4%	29.7%	91.7%	45.9%	1.18	42.9%	13.18	83.0%	-0.55
A-5	20	5	130	11.92	54.5%	41.9%	96.9%	51.9%	0.16	31.6%	7.21	92.8%	-0.36
A-6	20	15	130	10.60	48.0%	30.3%	95.2%	41.6%	0.19	35.9%	12.16	89.4%	-0.38
A-7	20	5	170	15.89	72.6%	22.8%	78.5%	28.7%	1.31	47.4%	15.70	87.4%	0.28
A-8	20	15	170	10.87	50.2%	13.2%	62.9%	23.5%	1.77	41.0%	11.85	82.9%	0.46
A-9	12.5	10	150	13.25	60.9%	38.8%	94.3%	47.1%	0.39	38.0%	9.89	91.8%	-0.64
A-10	12.5	10	150	13.96	63.0%	40.1%	94.0%	46.4%	0.41	39.8%	12.20	95.7%	-0.55
A-11	12.5	10	150	13.25	60.6%	36.6%	94.2%	44.1%	0.29	37.4%	9.41	86.8%	-0.50

a AC, Acid loading (mg/100g dm); t, Time (min); T, Temperature (°C).
b XOS percentage of total xylan equivalent recovered
c ArOS percentage of total arabinan equivalent recovered
d Combined acetic acid, formic acid, furfural and HMF
e Total dissolved solids hydrolysate
f Dissolved solids excluding quantified oligomers, sugars and inhibitors

Table 4A-3 Results for acid catalysed XOS production for BSG-SPD

Run	Independent variables[a]			XOS (g/l)	XOS yield (wt%)	ArOS yield (wt%)	XOS%[b] (wt.%)	ArOS%[c] (wt.%)	Inhibitors[d] (g/100g)	TDS[e] yield (wt.%)	TDS ND[f] (g/100g)	Mass balance closure	CSF
	AC	t	T										
B-1	5	5	130	13.66	27.0%	29.1%	96.3%	64.2%	0.12	21.2%	6.94	91.1%	-1.31
B-2	5	15	130	17.44	33.8%	35.1%	96.9%	64.0%	0.28	24.8%	7.08	85.2%	-1.19
B-3	5	5	170	31.65	65.5%	35.1%	93.0%	48.9%	1.14	34.1%	8.97	86.9%	-0.13
B-4	5	15	170	27.08	56.0%	17.9%	86.3%	39.5%	1.29	32.8%	9.66	87.3%	0.14
B-5	20	5	130	20.59	42.1%	27.6%	91.3%	34.2%	0.31	27.2%	8.05	83.7%	0.08
B-6	20	15	130	24.76	50.9%	26.8%	89.3%	29.1%	0.44	30.6%	8.12	86.5%	0.27
B-7	20	5	170	16.58	33.4%	10.1%	56.3%	21.9%	2.51	36.6%	14.94	90.1%	0.99
B-8	20	15	170	7.25	15.0%	3.8%	38.8%	14.3%	3.09	25.4%	8.49	78.4%	1.07
B-9	12.5	10	150	27.75	55.7%	29.3%	90.2%	36.4%	0.89	34.1%	10.55	88.0%	0.04
B-10	12.5	10	150	28.18	56.0%	22.5%	89.3%	28.7%	0.99	35.0%	10.76	88.8%	0.03
B-11	12.5	10	150	29.38	59.0%	28.6%	90.7%	36.4%	0.87	35.6%	11.05	88.1%	0.07

a AC, Acid loading (mg/100g dm); t, Time (min); T, Temperature (°C).
b XOS percentage of total xylan equivalent recovered
c ArOS percentage of total arabinan equivalent recovered
d Combined acetic acid, formic acid, furfural and HMF
e Total dissolved solids hydrolysate
f Dissolved solids excluding quantified oligomers, sugars and inhibitors

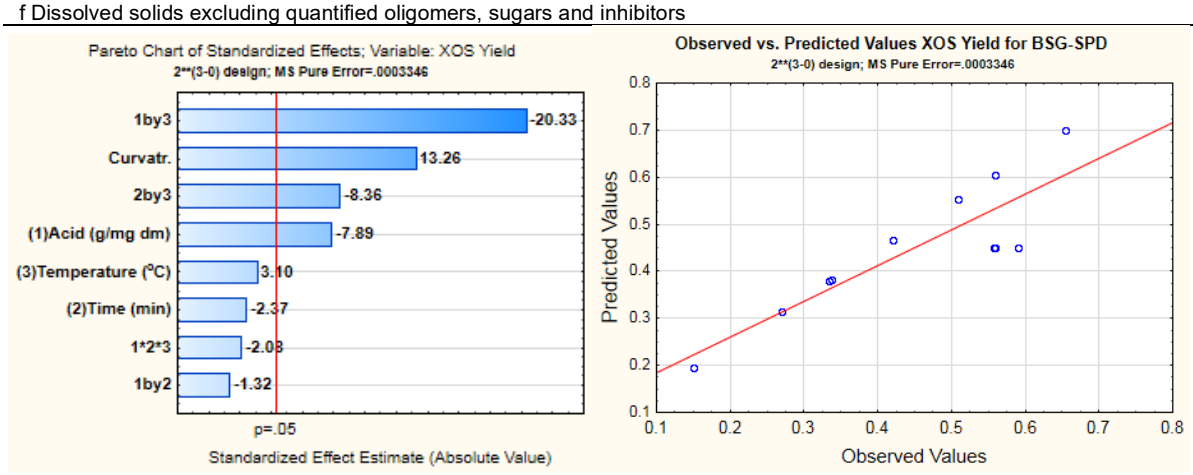

Figure 4A-1 Analysis of factorial results for BSG-SPD: standardised effects and regression model for XOS yield from BSG-SPD

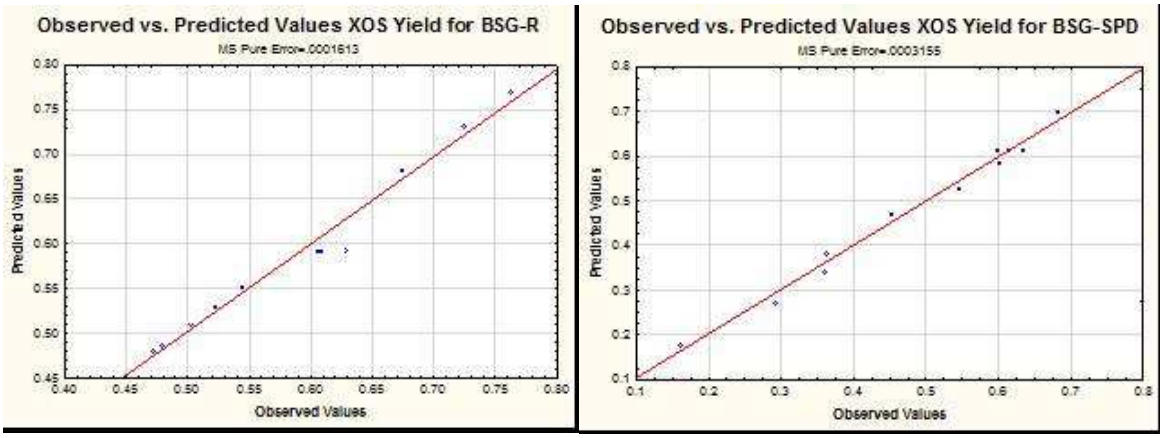

Figure 4A-2 Model response prediction for XOS yield vs. observed values (A) BSG-R and (B) BSG-SPD with quadratic term

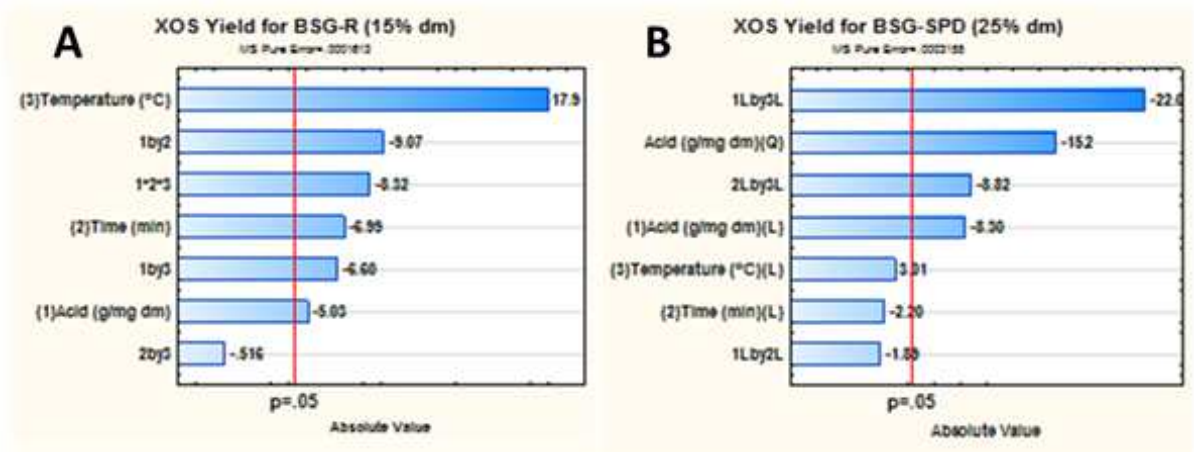

Figure 4A-3 Pareto chart of standardized effects, XOS yield (A) BSG-R (B) BSG-SPD

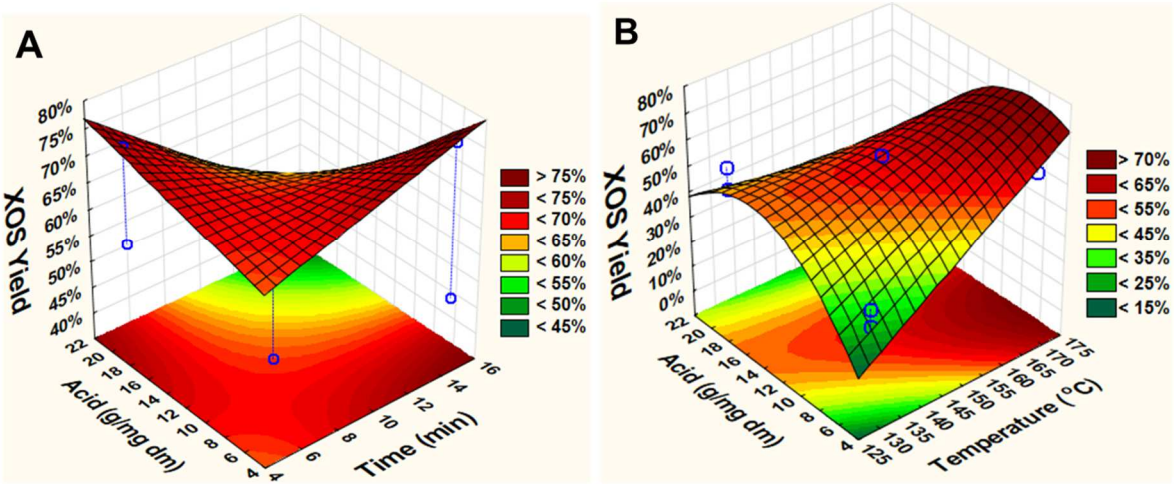

Figure 4A-4 Effect of acid loadings and treatment time in ELA HTT at 170 °C of BSG with XOS yields represented in surface plots for (A) BSG-R and (B) BSG-SPD with quadratic effect

Table 4A-4 Regression coefficients and R2 assessing the correlation and significance of the fitted modelsa

	BSG-R			BSG-SPD		
Regression coefficients	XOS yield (wt.%)	Inhibitors (g/100g)	TDS yield[b] (wt.%)	XOS yield (wt.%)	Inhibitors (g/100g)	TDS yield[b] (wt.%)
a_0	0.59267	-0.74538	0.83956	0.56891	0.91688	0.34891
a_1	-0.04524	-0.11249	-0.06177	-0.10211	0.88063	0.01722
a_2	-0.06283	-0.13141	-0.06098	-0.03074	0.25273	-0.01384
a_3	0.16135	0.00537	-0.00277	0.04014	1.71882	0.06249
a_{12}	-0.08149	-0.00016	0.00500	-0.01720	0.10217	-0.02525
a_{13}	-0.05932	0.00090	0.00040	-0.26305	0.70171	-0.04189
a_{23}	-0.00464	0.00106	0.00038	-0.10819	0.11189	-0.04883
a_{123}	-0.07478	0.00000	-0.00003	0.00000	0.00000	0.00000
a_{11}	0.00000	0.00000	0.00000	-0.32865	0.46030	-0.11617
R^2	0.97784	0.92095	0.98125	0.99146	0.99613	0.95160

[a] ANOVA for XOS concentration and yields models given
[b] Total dissolved solids yield in hydrolysate

Table 4A-5 Analysis of variance of the fitted models for XOS yield from BSG-R

	SS[a]	d.f.[b]	MS[c]	F-value	p-value
XOS yield (wt.%)					
AC	0.0040928	1	0.004093	25.38037	0.037215
t	0.0078949	1	0.007895	48.95843	0.019820
T	0.0520682	1	0.052068	322.8886	0.003083
AC*t	0.0132805	1	0.013281	82.35597	0.011926
AC*T	0.0070387	1	0.007039	43.64898	0.022152
t*T	0.0000430	1	0.000043	0.266683	0.656993
AC*t*T	0.0111830	1	0.011183	69.34848	0.014115
Lack of fit	0.0018441	1	0.001844	11.43555	0.077427
Pure Error	0.0003225	2	0.000161		
Total SS	0.0977677	10			

[a] sum of squares; [b] degrees of freedom; [c] mean of squares.

Table 4A-6 Analysis of variance of the fitted models for XOS yield from BSG-SPD

	SS[a]	d.f.[b]	MS[c]	F-value	p-value
XOS yield (wt.%)					
AC	0.020853	1	0.020853	62.3138	0.015672
t	0.001890	1	0.001890	5.6487	0.140629
T	0.003223	1	0.003223	9.6307	0.090032
AC*t	0.000592	1	0.000592	1.7676	0.315053
AC*T	0.138388	1	0.138388	413.5346	0.002409
t*T	0.023410	1	0.023410	69.9559	0.013995
AC^2	0.058916	1	0.058916	176.0547	0.005632
Lack of fit	0.001462	1	0.001462	4.3673	0.171812
Pure Error	0.000669	2	0.000335		
Total SS	0.249403	10			

[a] sum of squares; [b] degrees of freedom; [c] mean of squares.

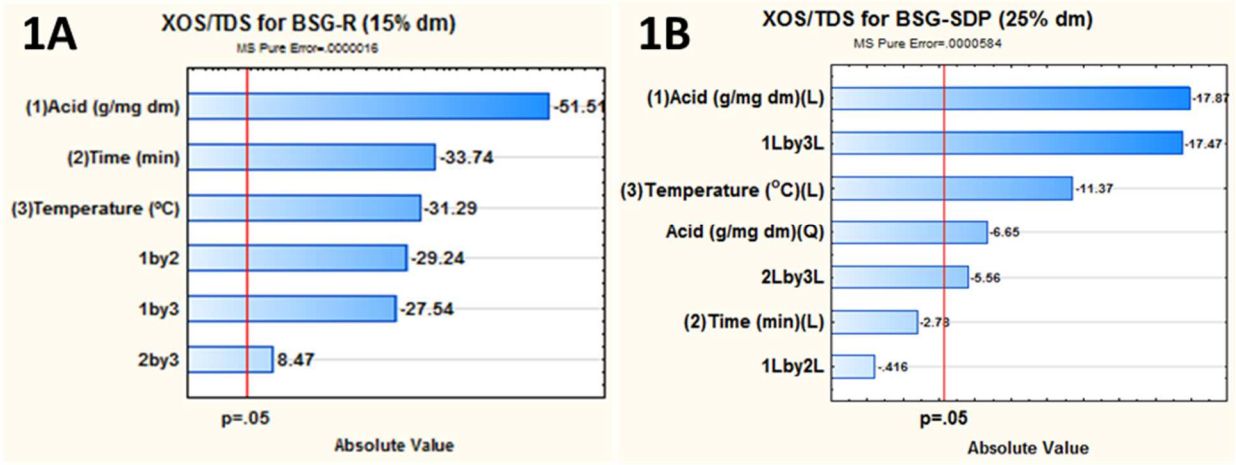

Figure 4A-5 Pareto standardised chart of effects for variable XOS/TDS (1A) BSG-R and (1B) BSG-SPD

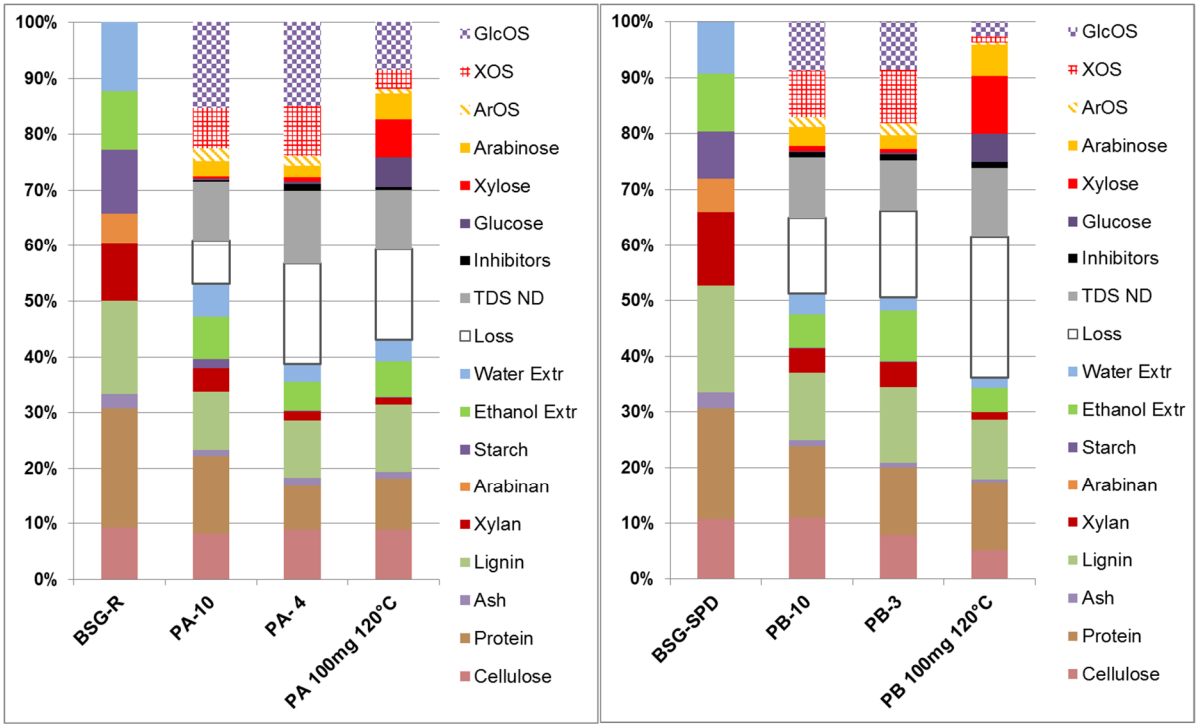

Figure 4A-6 Insoluble residue and hydrolysates obtained from hydrothermal treatments for (A) BSG-R and (B) BSG-SPD

Table 4A-7 Protein analysis from BSG, press liquid and HTT solid residue

Amino acid (wt.%)	Feedstock		Solid Residue	
	BSG-R	Screw press liquid-solid filtrate	A-4 170°C 15min 5mg	B-3 170°C 5min 5mg
Basic amino acids				
Arginine	6.5%	5.6%	3.1%	3.3%
Histidine*	3.2%	1.5%	2.8%	2.7%
Lysine*	2.3%	3.5%	0.9%	1.0%
Subtotal	12.0%	10.5%	6.9%	7.0%
Hydrophobic amino acids				
Alanine	4.5%	4.8%	5.2%	5.4%
Glycine	4.9%	4.4%	4.8%	5.0%
Isoleucine*	3.7%	4.1%	4.2%	4.7%
Leucine*	7.8%	7.8%	9.1%	9.4%
Methionine*	2.4%	2.2%	2.8%	2.4%
Phenylalanine*	7.5%	5.1%	8.7%	8.0%
Proline	7.2%	12.2%	7.3%	7.3%
Valine*	5.8%	5.4%	6.3%	6.5%
Subtotal	43.8%	46.0%	48.4%	48.7%
Hydrophilic amino acids				
Asparagine	6.3%	7.3%	5.2%	5.9%
Glutamine	23.2%	24.4%	24.5%	23.8%
Serine	5.8%	4.6%	5.6%	5.5%
Threonine*	4.6%	3.8%	4.6%	4.7%
Tyrosine	4.1%	3.4%	4.9%	4.4%
Subtotal	44.2%	43.4%	44.8%	44.3%
Essential amino acids*				
Subtotal included	37.3%	33.4%	39.5%	39.3%
Total amino acids[a]	23.3%	43.0%	25.9%	22.2%
Crude Protein	23.4%	39.5%	26.5%	23.3%

a Tryptophan and Cysteine not determined

5. Pilot scale xylo-oligosaccharide production through steam explosion of screw press dried brewers spent grains

Contribution to overall scope of work

BSG hemicellulose fractionation techniques by LHW hydrothermal treatment (HTT) of a Weiss BSG (WBSG) was first investigated in Chapter 4. A baseline for comparison was established by treating raw WBSG (15% dm) and screw press dewatered WBSG (25% dm) at literature reported optimal LHW HTT conditions (11% dm) for maximum XOS and xylose production in a similar bench-scale batch stirred Parr reactor. Results from autocatalytic and extremely low acid (ELA) dosed HTT showed >15% dry matter content in HTT can lead to similar or higher XOS and hemicellulose yields at reduced process temperatures. Therefore, the next consideration was to scale up the HTT of BSG in an appropriate high solid loading HTT system, i.e. pilot-scale steam explosion (SE) system.

To scale up the XOS production from BSG in steam explosion HTT in Chapter 5, process conditions were tested considering results from Chapter 4 and optimisation studies in LHW from literature. From Chapter 4, 180 ºC and 15 min, was selected which in autocatalytic LHW HTT showed highest XOS yields (78.0%) using WBSG (15% dry matter). As the preferred process condition, 180 ºC and 10 min, was selected based on the maximum XOS + ArOS yield reported in multivariate optimisations in LHW HTT of BSG (11% dry matter). Using severity factor (SF), the process conditions of 200 ºC and 5 min (SF of 3.65) was selected from reported maximum XOS +ArOS yield (77%) obtained at 195 ºC (SF of 3.65) in nonisothermal LHW HTT optimisations in stirred batch reactors (11% dry matter). An additional malt BSG type, PBSG, was considered and both WBSG and PBSG were dewatered before SE HTT with a screw press, effectively creating three moisture levels, raw 15% dm and screw pressed 25 and 32% dry matter. Results from the SE HTT show significant process intensification was achieved with XOS yields of 75.3 and 73.1% obtained for WBSG and PBSG respectively, both with 25% dm at 180 °C and 10 min treatments. That said, in Chapter 4, results from the bench-scale Parr reactor with WBSG (15% dm) achieved a similar XOS yield (78.0%), nevertheless, XOS yields in SE were obtained using 47% less water compared to results with WBSG (15% dm) from the bench-scale Parr reactor. Additionally compared to literature reports with similar XOS yields at 190 ºC to 200 ºC, the process intensification achieved an above 60% reduction in water and reduced process temperatures to 180 °C.

Moreover, SE was more suitable for HTT of BSG with 25% dry matter content compared to the stirred bench-scale Parr. The total xylan equivalent yield (79.4-81.1%) at the conditions

for the highest XOS yield in pilot-scale steam explosion treatment using no added catalyst, was higher compared to the ELA HTT in the bench-scale Parr (70.3%). The higher XOS yield and xylan recovery in SE HTT with 25% dry matter content BSG were achieved due to reduced formation of degradation products (0.81 g/100 g dm), compared to the Parr (1.14 g/100 g/dm) using the same WBSG. Adjusting SE process conditions to 200 °C and 5 min increased the fraction of the valuable xylobiose (X2) and xylotriose (X3) in the XOS to 27.0%, from 7.5%, at the highest XOS yield process condition (180 ºC 10 min). On the other hand, the addition of 35 mg SO_2/g dm at the process conditions for the highest XOS yield increased the X2 and X3 fraction to 25.3%.

Furthermore, acidifying effect (mol H+ per gram dry BSG) in SE HTT, from the screw press dewatering of wet 15% dm WBSG to 25% dm and 32% dm, was found to be proportional to the change in moisture content alone when assessed by comparison with air-dried BSG. This suggests that the screw press dewatering can be considered a water reduction method and the effect on autocatalytic SE HTT of BSG can be related to the change in moisture content alone. This is in agreement with autocatalytic LHW HTT results obtained in the Parr reactor from Chapter 4.

In conclusion, the second part of the first overall research objective required responses to be characterised from steam explosion for two types of BSG, both raw and screw press dewatered. This was done by using reported bench-scale multivariate optimised LHW HTT process conditions as a near optimum for steam explosion scale-up. Confirmation runs were conducted in triplicate and additional runs were conducted to establish time and temperature effects from the chosen condition. The resulting hydrolysate pH was used to compare and assess the effect of the screw press dewatering on the SE HTT by calculating the mol H+ released per gram dry BSG.

The results from this Chapter 5 for the steam explosion HTT of 25% dm BSG at 180 ºC and 10 min was implemented in Chapter 6 for the techno-economic evaluation of XOS (Scenario A) and xylitol (Scenario B) production from BSG.

- Hydrothermal treatment results from this preferred confdition including yields of all sugars and oligomers were used in the process models. The composition of the remaining solids after treatment were also incoprporated in the process model including protein, lignin and ash.
- Screw press component fractionation data in the pressed solid and liquid (suspended and soluble) from this chapter was used in Chapter 6.

Pilot scale xylo-oligosaccharide production through steam explosion of screw press dried brewers spent grains

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Authors' contribution

LJ is the primary author and investigator of the paper, conceptualised the approach for scale-up in steam explosion, performed the screw press dewatering and steam gun experiments and the analysis of the results. EvR gave technical guidance during the research and write-up, was the internal reviewer and assisted in the structuring of the paper. JFG is the study leader and contributed to draft the final manuscript. All authors provided input to the manuscript and read and approved the final manuscript.

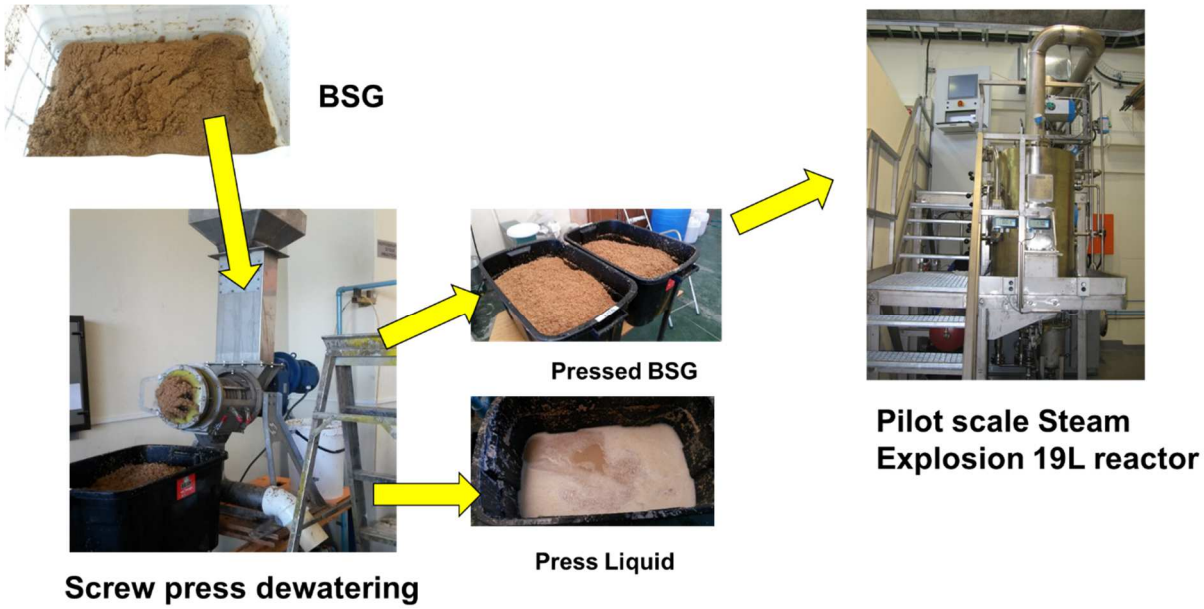
Abstract

Brewers' spent grains (BSG) represent the largest quantity of solid waste from brewing, while xylooligosaccharides (XOS) produced from BSG show promising applications in food, beverage and health products. Production of XOS form a Weiss and malt BSG was scaled-up in steam explosion hydrothermal treatment using process conditions from bench-scale liquid hot water optimisations in stirred batch reactors. Three levels of moisture (15-25-32% dry matter) achieved by screw press dewatering were evaluated, with changes in treatment temperatures and times. Results show the highest XOS yields (>73.1%) were obtained, for both BSG's, at process condition selected (180 ºC, 10 min) with 25% initial dry matter content. These yields were higher than reported bench scale optimisations (61%), but obtained using 60% less water, hence initial dry matter content was an important variable affecting XOS yield. Inexpensive bench-scale optimisations can provide cost-effective near optimal operating conditions for use in pilot-scale steam explosion.

Brewer's spent grains; steam explosion; Xylo-oligosaccharides; screw press drying; high solids loading

Highlights

- High solids loading (15-25-32% dry matter) BSG hydrothermal treatment
- Simple screw press dewatering for XOS production intensification
- Scale-up to pilot steam explosion by using reported optimal conditions from bench stirred batch reactors



5.1 Introduction

Brewers' spent grains (BSG) are the leftovers of barley malt, wheat, and rice used in beer production, after saccharification of the grains' starch content. Worldwide, around 40 million tonnes of BSG is produced annually (Lynch, Steffen and Arendt, 2016), and it comprises of up to 85% of the solid waste produced by breweries (Nigam, 2017). BSG has a high moisture content (~75-85%) and is typically disposed of without drying, mostly as a cheap ruminant feed supplement (Mussatto, 2014; Kerby and Vriesekoop, 2017). Regulations regarding animal feeds are becoming increasingly stringent, and compel breweries to consider alternative solutions for BSG disposal and valorisation (Kerby and Vriesekoop, 2017).

Recently, higher-value food product applications from processed BSG have been reported such as health-promoting, indigestible, soluble polysaccharides, i.e. xylo-oligosaccharides (XOS) (Lynch, Steffen and Arendt, 2016; Ikram *et al.*, 2017). BSG is reported to be well suited for the production of XOS since it contains fibre (*circa* 70%) that is high (by up to 40%) in xylan polymers (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). Xylan is the major component of the hemicellulose fraction in BSG and is more amorphous and accessible than cellulose or lignin (Ibbett *et al.*, 2011; Gomez *et al.*, 2015). Thus, xylan can be fractioned through selective solubilisation and depolymerisation by hydrothermal treatment (HTT) to produce XOS of varying degrees of polymerisation (DP) and substitution with arabinan, uronic acid, phenolic and acetyl groups (Carvalheiro et al., 2004; Gómez et al., 2015; Gullón et al., 2011). Short-chain (DP<10) XOS are valued for their prebiotic effect, in particular XOS substituted with arabinan or arabino-oligosaccharide (ArOS) (Gomez *et al.*, 2015).

Hydrothermal treatment is the most widely researched and applied technology for lignocellulosic biomass deconstruction, fractionation and conversion into constituent sugars and other components (Wyman et al., 2005). HTT is classified as a physiochemical treatment method that mainly uses the hydrolysis process through the action of H+ ions in liquid water or saturated steam at temperatures between 120 °C to 250 °C (Bayer, 2007; Ibbett *et al.*, 2011). In HTT, glycosidic bonds in the xylan polymers are cleaved through hydrolysis by H+ ions (Garrote, Domı and Parajo, 2004; Agbor *et al.*, 2011) and the H+ concentration affects the xylan depolymerisation rate proportionally (Mosier, Ladisch and Ladisch, 2002). The HTT technology is widely described using a severity factor, $\log(R_0)$, a combination of process variables temperature and time (Overend, Chornet and Gascoigne, 1987) or in a combined severity factor (CSF), $\log(R'_0)$ that also includes the final pH of the hydrolysate (Chum *et al.*, 1990). Importantly, solids loading is not present in either description of severity although, as demonstrated here, it may affect the final pH

Autocatalytic HTT uses mainly the weak acid properties of water at elevated temperatures and that is accelerated by acetic, phenolic and furanic acids formed during solubilisation and depolymerisation of hemicellulose and lignin (Pedersen and Meyer, 2010; Pedersen, Johansen and Meyer, 2011). Autocatalytic HTT can be enhanced with an addition of acid catalysts to selectively increase xylan solubilisation and depolymerisation rate coefficients at lower temperatures. This can result in improved product yields and recoveries through a reduction in the formation of temperature-sensitive of degradation products, by lowering their rate coefficients (Shen and Wyman, 2011; Gurgel, Pimenta and Curvelo, 2014).

In HTT, the concentration of degradation products and the product yields can be significantly affected by the dry matter content, i.e. the inverse of the moisture content of the biomass in the reaction (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Ibbett *et al.*, 2011; Sui and Chen, 2015). Although higher dry matter contents during HTT is desired for higher product concentrations and lower process heat requirements (Modenbach and Nokes, 2012), a significant reduction of xylan yields is commonly reported for increased dry matter content when using lignocelluloses other than BSG (Stephen Glen Allen *et al.*, 2001; Yang *et al.*, 2004; Ibbett *et al.*, 2011; Modenbach and Nokes, 2012). However, some studies reported that increased dry matter content in HTT can result in increased product and total xylan recovery (Jacobsen and Wyman, 2002; Cullis, Saddler and Mansfield, 2004; Mussatto and Roberto, 2006). Similarly, XOS yield increased from 34% at 10% dry matter, to 43% at 25% dry matter at 170 °C and the same treatment time in autocatalytic HTT of sugarcane bagasse (Vallejos *et al.*, 2012). These reports show that in XOS yield investigations in HTT, the dry matter content needs to be considered as an additional process variable to temperature and time.

HTT processes reported for lignocelluloses are applied, with or without catalysts, in various reactor systems such as stirred batch (Gomez *et al.*, 2015), stationary flow-through (Yang *et al.*, 2004), plug flow (Church and Wooldridge, 1981) or continuous process mode (Heitz *et al.*, 1991), while using steam injection, conductive or microwave heating (Roos *et al.*, 2009). The reactions take place mainly in the liquid water phase, such as in stirred (Gomez *et al.*, 2015) or unstirred batch vessels (Vallejos *et al.*, 2012), single phase steam (Stephen Glen Allen *et al.*, 2001) or in mixed liquid water and vapour phase such as in steam explosion (Brownell and Saddler, 1987; Bayer, 2007; Roos *et al.*, 2009; Lischeske *et al.*, 2016). Various terms are reported that describe these HTT process configurations and their applications, such as liquid hot water (LHW), hot water, pressure cooking by water, compressed hot water, autocatalytic HTT, hydrolytic processing, hydrothermolysis, water pre-hydrolysis, pre-treatment, steam treatment, steam-aqueous treatment and steam explosion (SE)/gun (Bayer, 2007). Despite the different modes of operations and reactor configurations from all these HTT processes,

the common roles of water in these reactions are (i) the dominant heat transfer medium; (ii) to physically mobilize the polymers in biomass cell walls; (iii) to be a co-reactant in activated intermediates; and (iv) is a solvent for reactants and products (Brownell and Saddler, 1986; Cullis, Saddler and Mansfield, 2004; Yang *et al.*, 2004; Ibbett *et al.*, 2011). It is therefore possible that some of these different systems are able to produce comparable results with appropriate process conditions (Lischeske *et al.*, 2016).

Early comparisons between different reactor configurations for HTT using LHW and SE of wood chips reported similar xylan removal results, but the recoveries were higher for LHW HTT (Brownell and Saddler, 1987; S G Allen *et al.*, 2001; Laser *et al.*, 2002). More recently Roos et al., (2009) reported that the product recovery from HTT of barley husks in LHW (8.5 g sample at 8.5% dm), using microwave heating, could be reproduced at larger reactor scales by using the same treatment severities (larger 500 g at >30% dm) in pilot-scale SE. A more comprehensive comparison of HTT of cornstover was conducted in 4 reactor configurations (batch flow-through, LHW batch stirred, SE and continuous screw reactor), from 3 g batch (10% dry matter) up to 500 kg/d (30% dry matter) continuous screw systems (Lischeske *et al.*, 2016). The optimum HTT process conditions for xylan yield identified through multi-variate optimisations from the smaller, less costly bench batch reactors were within the near-optimal range of process conditions of the largest pilot-scale reactor systems (Lischeske *et al.*, 2016). The maximum total yield of xylan obtained by the smallest batch system (78.9%) was within a two standard deviations of the maximum obtained in the other larger reactor systems (Lischeske *et al.*, 2016). Therefore, process conditions for XOS production from bench-scale optimisation using homogeneous low solids HTT could be used as possible near optimal process conditions in other systems such as SE.

Steam explosion (SE) is widely reported as an efficient HTT method specifically to maximise the production of XOS from barley husk (Roos *et al.*, 2009), corncobs (Wang *et al.*, 2013) and bagasse (Carvalho *et al.*, 2018); however, no such optimisations of XOS production from BSG are reported. Recent SE HTT studies of BSG focus on improving digestibility (Qihua *et al.*, 2010; Kemppainen *et al.*, 2016). Another study optimised total xylan recovery (Rojas Pérez, 2018). In the study a maximum of 47% xylan yield (33.5% XOS yield) was reported at 173 °C and 15.5 min. The best XOS yields reported from BSG are from optimisations in autocatalytic LHW HTT studies in stirred batch reactors with less than 11% dry matter, with a maximum XOS yield of up to 77% in nonisothermal LHW HTT up to 200 °C (Gomez *et al.*, 2015) and *ca* 61% from isothermal (*ca* 190 °C and 5 min) LHW HTT optimisations (Carvalheiro *et al.*, 2004; López-Linares *et al.*, 2019). However, Gómez et al., (2015) showed isothermal HTT at even lower temperatures, with equivalent severity, T = 180 °C for t = 12.2 min (severity factor = 3.65), increased the overall yield of XOS substituted with arabinan (ArOS). Therefore, this

preferred process condition for BSG for production of XOS high in ArOS can be used as a guide for a scale up in more efficient high solids pilot SE.

In order to investigate SE at higher solids loadings than previous studies, an appropriate pilot-scale (and industrial) method of dewatering had to be found. Mechanical dewatering of BSG with a screw press was selected on the basis of several process benefits ('Patent WO1999023260A1 - Method for proc...o produce pentose Blinkov 1999.pdf', no date; Ishiwaki *et al.*, 2000): (i) reduced energy requirements compared to conventional drying, (ii) removal of the protein fraction into a separate by-product and (iii) increase in the fibre fraction of the residual BSG, which is then more suitable for XOS production. Additionally the screw press could enhance the SE HTT for XOS production by three mechanisms. Firstly, the mechanical shear action of the screw press can cause defibrillation and shortening of fibres of the biomass, resulting in increased surface area (Yan *et al.*, 2014). Secondly, screw press dewatering can increase acidification of HTT of the biomass by the reduced acid neutralisation (buffering) capacity in the pressed biomass, as a result of removed components in the water fraction, such as soluble salts or ash (Jacobsen and Wyman, 2000) and reduction of the biomass proteinaceous compounds (Liao *et al.*, 2004). Thirdly, screw press dewatering can increase the rate of hemicellulose depolymerisation by acidification in HTT by way of increasing H+ concentration with the water reduction in biomass (Mosier, Ladisch and Ladisch, 2002).

Therefore, the impact of screw press dewatering on the XOS yield obtained from subsequent autocatalytic SE HTT was investigated using two types of BSG, namely, Weiss and pure pale malt brew (WBSG and PBSG). Three levels of moisture contents were applied in such SE HTT, i.e. raw BSG (15% dry matter) and screw pressed BSGs at 25 and 32% dry matter, for both types of BSG. The pH values of the resulting hydrolysates were also used to compare acidification in the SE HTT between the different BSG types and screw press moisture levels, as based on the mol H+ per gram dry BSG. Additionally, air dried BSG (90% dm) was used to benchmark for acidification in SE.

Besides autocatalytic SE treatments of the various BSGs, the acidification and the depolymerisation rate of xylan were also enhanced by the addition of external catalysts. SE treatments of BSG with SO_2 catalyst was done at the conditions for highest XOS yield to compare XOS yields, and yields of short chain XOS oligomers xylobiose (X2) and xylotriose (X3) in the XOS hydrolysate. In SE acid catalysts H_2SO_4, SO_2 or CO_2 gas are commonly used to obtain mainly monomeric sugars (Öhgren, Galbe and Zacchi, 2005; García-Aparicio *et al.*, 2011; Rojas Pérez, 2018); however, their addition can be used to vary the XOS yields and DP of the XOS (Carvalho *et al.*, 2018). In this study direct catalyst dosing of BSG was done with SO_2 prior to SE using a 4.5 wt.%. SO_2 solution and solid potassium metabisulphite ($K_2S_2O_5$)

or PMB a common additive in food and wine as a source of SO_2 (Barata *et al.*, 2008; Corte *et al.*, 2012).

5.2 Materials and methods

5.2.1 Raw material and screw press

BSG was sourced from a local brewery in Newlands (Cape Town, South Africa) in fresh 1 m^3 lots, either as a single brew from a WBSG recipe consisting of 50/50 barley/wheat, or a PBSG made of a single brew from a pure malt recipe. A 2.2 kW single screw press (NEW Eco-tec Verfahrenstechnik GmbH, Germany) with a screen cage of 0.3 m long x 0.15 m diameter and 0.6 mm slotted opening, was used to reduce the moisture contents in the two types of BSG. An adjustable opening at press outlet allowed for a measure of flow control, was used to regulate the extent of pressure build-up in the screw-press as well as the resulting water removal and dry mass content of the solid product. Triplicate runs were conducted to compare the screw press operation with respect to flow rate of each BSG type and the resulting moisture contents. Selected feed samples were taken randomly of raw feedstocks and pressed products (25 and 32% dry matter), aliquoted in sealed vacuum bags and stored frozen at -20 °C until required. Samples were defrosted in a water bath at 25 °C before use (Bartolomé et al., 2002).

5.2.2 Steam explosion fractionation of BSG

SE experiments were carried out in a pilot-scale 19 L capacity “steam gun” system that is well described elsewhere (García-Aparicio *et al.*, 2011). BSG was loaded manually at a top of a cylindrical high-pressure reactor vessel, and saturated steam from an electrical boiler was used to heat the material to the required temperature. An explosive flash discharge cooled and released the BSG hydrolysate slurry into a flash tank once the required treatment time had been reached. The slurry of residue was collected from the flash tank and centrifuged to separate a liquid fraction for analysis. The resulting solids fraction was washed with distilled water and stored at -20 °C before analysis. The SE reactor vessel was preheated to the required temperature by saturated steam and loaded with a sample of 1 kg dry mass (wt.%) of BSG.

SE treatment times and temperatures of the two BSG feedstocks are given in Table 5-1; for WBSG (runs A1-A12) and for PBSG (runs B1-B7). These HTT conditions used include, from Chapter 4, 180 ºC and 15 min, which showed highest XOS yields (78.0%) using WBSG (15% dry matter). Preferred process conditions were selected from reported maximum XOS + ArOS yield: (i) 180 ºC and 10 min from multivariate optimisations in LHW HTT (11% dry matter) for maximum XOS + ArOS yields and (ii) 200 ºC and 5 min (SF of 3.65) from nonisothermal LHW

HTT optimisation in stirred batch reactors (11% dry matter) at 195 ºC (SF of 3.65). Catalysed SE HTT runs (A-11/12 and B-7) with SO_2 were conducted at process conditions for highest XOS yield obtained in uncatalysed SE. To achieve the desired SO_2 loading in the BSG, a 4.5 wt.% SO_2 was added in BSG prior to SE (runs A-11 and B-7), however in run A-12 potassium metabisulphite ($K_2S_2O_5$) was added (57% wt.% equivalent SO_2) to reach the 30 mg equivalent SO_2/g dry feedstock (Table 5-1). Air dried feedstock (WBSG-90%) was also prepared for run A-9 from WBSG-25% for SE benchmarking control run A-7.

Table 5-1 Steam explosion runs for WBSG and PBSG

Feedstock type used		Treatment parameters		
WBSG	PBSG	Starting dry mass (wt.%)	Temperature (°C)	Time (min)
A-1	B-1	15	180	10
A-2		15	180	25
A-3		25	150	25
A-4	B-2[c]	25	180	10
A-5	B-3	25	180	15
A-6		25	180	25
A-7	B-4	25	200	5
A-8	B-5	32	180	10
A-9	B-6	32	200	5
A-10[a]		90	200	5
A-11	B-7	25	180	10
25 mg SO_2	35 mg SO_2			
A-12		25	180	10
30 mg SO_2 eq[b]				

[a] WBSG-25% air dried to 90% dry matter
[b] SO_2 loaded with $K_2S_2O_5$ addition
[c] Run conducted in triplicate

SE HTT results of hemicellulose, gluco-oligosaccharide (GlcOS), XOS, arabino-oligosaccharides (ArOS), monomeric sugars, degradation products/inhibitors (acetic acid, formic acid, hydroxymethylfurfural (HMF) and furfural) were used to characterise the effects of screw press dewatering, SE treatment time and temperature; these were compared to bench-scale optimisation obtained under LHW HTT conditions from literature (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015; López-Linares *et al.*, 2019) and (Chapter 4). GlcOS, XOS and ArOS yields after SE treatment were calculated as the weight fraction of each of the starting polysaccharides (glucan, xylan or arabinan, depending on which yield) in the feedstock BSG recovered in the liquid hydrolysate as oligosaccharide (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015). Hemicellulose yield was calculated as the fraction of the starting amount of hemicellulose that was recovered as pentose sugars and oligosaccharides in the liquid products. Inhibitor yields were calculated as mass yields relative to the dry mass starting feedstock. The total oligosaccharide equivalent weight recovered is the mass equivalent of reducing sugars and oligosaccharide recovered in the hydrolysate liquid after a treatment (Vallejos *et al.*, 2012). Yields of xylobiose (X2) and xylotriose (X3) were calculated as a fraction of the XOS in the hydrolysate. The oligosaccharide yields relative to reducing sugars yield (GlcOS%, ArOS%, and XOS%) were calculated as the mass oligosaccharide recovered,

relative to sum total of oligosaccharide and reducing sugars as equivalent mass oligosaccharide recovered (TX_eR).

Additionally, a combined severity factor (CSF) was calculated for SE HTT, using the resulting pH in the slurry product, for comparison of HTT results. The CSF is $\log R'_0 = \log R_0 - pH$; where $R_0 = t.\exp((T_r - 100)/14.75)$ and that combines time (t) and temperature (T_r), and incorporates pH to include the contribution of added catalysts on biomass HTT (Chum *et al.*, 1990). The contribution of increased dry matter content to changes in pH's with HTT was considered using the resulting ratio as R[H+], of mol H+ per gram BSG at the same process condition from one dry matter content with another.

5.2.3 Analytical methods

Compositional analysis of all solids, i.e. raw feedstock, screw press products, residue from SE HTT and its moisture content, were determined according to standard laboratory analytical procedures (LAP) for biomass analysis from National Renewable Energy Laboratory (NREL, USA) (Sluiter *et al.*, 2010). Bulk densities of all feedstocks were determined by measurement in a graduated cylinder according to QAS/11.450 (WHO, 2012). BSG total starch and residual starch were determined before and after NREL extractives using a starch kit from Megazyme (K-TSTA, Ireland) (Robertson, I'Anson, *et al.*, 2010). Nitrogen content of samples was determent by crude nitrogen determination by the Kjeldahl method (D-3 Velp, Italy), where N x 6.25 was applied (Pires *et al.*, 2012). Amino acids were determined using a Waters Acquity (Milford, USA) Ultra Performance Liquid Chromatograph (UPLC) separation with ultraviolet (UV) or fluorescence detection after derivation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC).

Liquid fractions were analyzed by high pressure liquid chromatography (HPLC) using an Aminex HPX-87H Ion Exclusion Column equipped with a Cation-H cartridge (Biorad, Johannesburg, South Africa), to determine the concentrations of short chain oligomers (X2 and X3), sugars (glucose, xylose, and arabinose) and inhibitors (acetic acid, formic acid, HMF and furfural) (García-Aparicio *et al.*, 2011). Additionally, oligosaccharide concentrations (GlcOS, XOS and ArOS) were determined as the difference in the monomeric sugar concentration before and after acid hydrolysis (Carvalho *et al.*, 2018). Correction factors of 0.9 for pentose and 0.88 hexose to anhydrous oligomers were used (Vallejos *et al.*, 2012; Gomez *et al.*, 2015). All samples were analyzed at least in triplicate.

5.3 Results and discussion

Two types of BSG, from a malt (PBSG) and Weiss (WBSG) brew, were used as raw materials to investigate pilot scale SE HTT for XOS production. In particular, a screw press was used to dewater the BSG both from 15 to 25 and 32% dry matter contents prior to SE. A selection of

process conditions from optimised bench scale LHW HTT investigations for XOS production reported by (Gomez *et al.*, 2015) at 11% dry matter content, and confirmed at dry matter contents up to 25% (Chapter 4), were applied in the SE HTT. Results were evaluated for the extent of hemicellulose solubilisation from BSG and the recovery of XOS and associated oligosaccharides in the hydrolysate.

5.3.1 Dewatering of WBSG and PBSG with a screw press

5.3.1.1 Screw press operation in dewatering WBSG and PBSG

The throughput rate (kg/h) of the equipment decreased with increases in dry matter content of the resulting pressed BSG (Appendix Figure 5A-1). The energy requirement for processing PBSG from a starting 15% to 36.6% dry matter (maximum achieved) was 66.1 kWh/t BSG; similar to reported values (40 to 53 kWh/t BSG; (Weger *et al.*, 2014; Weber and Stadlbauer, 2017). Additionally, the type of BSG used in the screw press had a significant influence on the screw press operation, as the results show a higher throughput rate of 794.7 ±51.9 kg wet WBSG/h was achieved compared to PBSG (282.2 ±32.4 kg wet PBSG/h) to dewater the raw BSG to *circa* 25% dry matter content (Appendix Figure 5A-2). Literature reports that fine particles (0.2-0.8 µm) present in BSG from adjuncts, like corn grits, or fines in the malt, can affect screw press dewatering significantly by giving an elastic or dough-like property to the BSG (Huige, 1994).

5.3.1.2 Characteristics of screw press dried BSG product

Screw press dried solids from WBSG and PBSG, of *circa* 25% and 32% dry matter were selected in addition to the unpressed feedstocks (15% dm), effectively creating 6 different BSG's for subsequent use in SE treatment (Table 5-2). The screw pressing operation did not compact the press dried BSG, to the contrary, bulk density declined on a wet and dry basis (Table 5-2). On a dry basis, bulk volume reduced from 0.15 kg/l for wet WBSG-15% to 0.11 kg/l with WBSG-32%, which indicate an increased bed porosity and large void fraction created in the screw pressed material as a result of the removal of water, fines and soluble materials.

The compositional analysis show total starch content on a dry-mass basis in raw WBSG-15% was 12.9%, nearly 3 times that of PBSG-15% (Table 5-2). The screw press operation reduced total starch in both types of BSG (by *circa* 33% in WBSG-25% and PBSG-25%), though WBSG-32% still contained 7.6% total starch. The reduction of starch is a result of the selective removal of fine suspended starch particles and soluble starch within the large water fraction removed with the press liquid (Stiles and Herbert, 1977; Jay *et al.*, 2008). Together with the starch, fine particles high in protein were removed with the press liquid fraction in the screw dewatering of raw PBSG and WBSG, which decreased the crude protein for the solid press products (Table 5-2). The fine insoluble particles in the press liquid removed from WBSG and

PBSG showed 35.5 and 46.5% crude protein content (43.0 and 50.6% amino acids), respectively, obtained at 25% dry matter pressings (solid residues are compared in section 5.3.4.2). The lowering of starch and protein content with the screw press dewatering, increased the lignocellulose and hemicellulose fractions present in both types of BSG; hemicelluloses increased in both WBSG (18.9 to 23.1 %) and PBSG (20.8 to 24.1%) (Table 5-2). Raw PBSG showed a higher starting xylan content (13.2%) compared to WBSG (11.7%), nonetheless screw pressing increased xylan fractions in both BSG's. Importantly, the screw press dewatering did not significantly change the composition of the BSG's since the values found for components of the 6 feedstocks from WBSG and PBSG are within ranges of other compositional values reported by Lynch et al., (2016) for BSG's, with hemicellulose 19.2-41.9%, for cellulose 0.3-33%, for starch 1-12%, for protein 14.2-31%, for lignin 11.5-27.8%, for lipids 3-13% and 1.1-13% for ash content.

Table 5-2 Main compositional changes in dry matter, starch, xylan and crude protein through screw pressing WBSG and PBSG

	Raw Unmodified				Screw Pressed 25%dm				Screw Pressed 32%dm			
	WBSG-15%		PBSG-15%		WBSG-25%		PBSG-25%		WBSG-32%		PBSG-32%	
Dry Mass %	**15.3**	*1.11*	**15.1**	*4.90*	**25.0**	*0.21*	**25.1**	*0.79*	**31.3**	*0.33*	**32.9**	*1.17*
Ratio Water to dry mass	5.5	:1	5.6	:1	3.0	:1	3.0	:1	2.2	:1	2.0	:1
pH	4.96		4.20									
Bulk Density												
Wet basis kg/l												
Compact	0.990	*0.050*	0.984	*0.023*	0.484	*0.038*	0.429	*0.038*	0.337	*0.004*	0.346	*0.011*
Loose	0.830	*0.035*	0.874	*0.072*	0.369	*0.016*	0.308	*0.011*	0.262	*0.004*	0.260	*0.012*
Dry basis kg/l												
Compact	0.151	0.013	0.148	0.048	0.121	0.009	0.108	0.009	0.105	0.002	0.114	*0.005*
Loose	0.127	0.011	0.132	0.044	0.092	0.004	0.077	0.004	0.082	0.002	0.085	*0.005*
Composition (db.)												
Extractives	**25.6**	**±**	**25.9**	**±**	**21.5**	**±**	**23.3**	**±**	**19.2**	**±**	**20.7**	
Water NREL	13.9	*0.04*	13.6	*0.02*	10.1	*0.03*	10.7	*0.08*	9.4	*0.03*	9.5	*0.06*
Ethanol NREL	11.7	*0.14*	12.3	*0.02*	11.4	*0.09*	12.5	*0.43*	9.8	*0.04*	11.2	*0.05*
Water soluble 25°C wash	11.5	*0.57*	9.6	*0.09*	8.8	*0.96*	6.0	*0.52*	5.6	*0.17*	4.2	*0.93*
Cellulose	**10.4**	*0.42*	**12.9**	*0.04*	**11.8**	*0.23*	**12.6**	*0.02*	**11.9**	*0.19*	**12.7**	*0.15*
Total Starch	**12.9**		**4.1**		**9.2**		**2.8**		**7.6**		**2.6**	
Starch	11.4	*0.17*	2.6	*0.16*	8.1	*0.53*	2.2	*0.13*	7.3	*0.20*	1.9	*0.19*
Maltodextrins	1.5	*0.00*	1.5	*0.00*	1.2	*0.00*	0.6	*0.00*	0.3	*0.00*	0.7	*0.00*
Hemicellulose	**18.9**		**20.8**		**21.8**		**22.7**		**23.1**		**24.1**	
Xylan	11.7	*0.10*	13.2	*0.43*	14.5	*0.05*	15.6	*0.11*	15.6	*0.19*	16.7	*0.27*
Arabinan	5.9	*0.21*	6.1	*0.30*	6.5	*0.17*	6.2	*0.18*	6.7	*0.06*	6.3	*0.03*
Acetyl groups	1.2	*0.00*	1.5	*0.63*	0.7	*0.20*	0.9	*0.10*	0.8	*0.09*	1.1	*0.08*
Lignin	**18.8**		**18.2**		**21.0**		**18.5**		**18.8**		**19.3**	
Acid soluble (AS)	5.3	*0.12*	6.0	*0.27*	6.7	*0.44*	5.8	*0.16*	6.9	*0.13*	3.9	*0.06*
Acid insoluble (AI)	13.4	*0.06*	12.3	*0.07*	14.3	*0.03*	12.7	*0.43*	11.8	*0.10*	15.4	*0.21*
Protein	**24.3**		**21.5**		**21.8**		**18.2**		**22.4**		**20.2**	
Crude protein	24.3	*0.35*	21.5	*0.08*	21.8	*0.65*	18.2	*0.30*	22.4	*0.06*	20.2	*0.08*
Amino acids	23.3		25.6									
Ash	**2.9**	*0.01*	**4.4**	*0.01*	**3.1**	*0.02*	**3.6**	*0.07*	**3.1**	*0.03*	**3.1**	*0.03*
Total	**113.8**		**107.8**		**110.3**		**101.7**		**106.2**		**102.8**	
Corrected Total*	**100.9**		**103.4**		**102.4**		**98.2**		**99.4**		**97.9**	

*Total adjusted for starch and protein in water and ethanol extracts

5.3.2 Pilot scale steam explosion HTT of WBSG and PBSG

The SE HTT process was applied as a method of BSG fractionation, investigated at pilot scale with the goal to maximise the production of XOS. A range of process conditions were tested (Table 5-1) that included: (i) process conditions (180 ºC and 15 min) chosen from bench scale LHW HTT using WBSG-15% (Chapter 4) for the highest XOS yield (78.0%) obtained; (ii) preferred process conditions (180 °C and 10 min) that was based on process conditions for maximising XOS +ArOS yield reported in LHW HTT optimisations using 11% dry matter BSG (Gomez *et al.*, 2015); and (iii) process conditions (200 ºC and 5 min) of equal severity (SF 3.65) as reported optimal nonisothermal (195 ºC) LHW HTT for maximum XOS + ArOS yield using 11% dry matter BSG (Gomez *et al.*, 2015). Both raw (15% dry matter) and screw pressed (25% and 32% dry matter) versions of these BSGs were tested in SE HTT and the results obtained were compared using combined severity factor (CSF) that incorporates the final hydrolysate pH which can better account for effects of dry matter content in HTT compared to a severity factor considering time and temperature alone (Chapter 4).

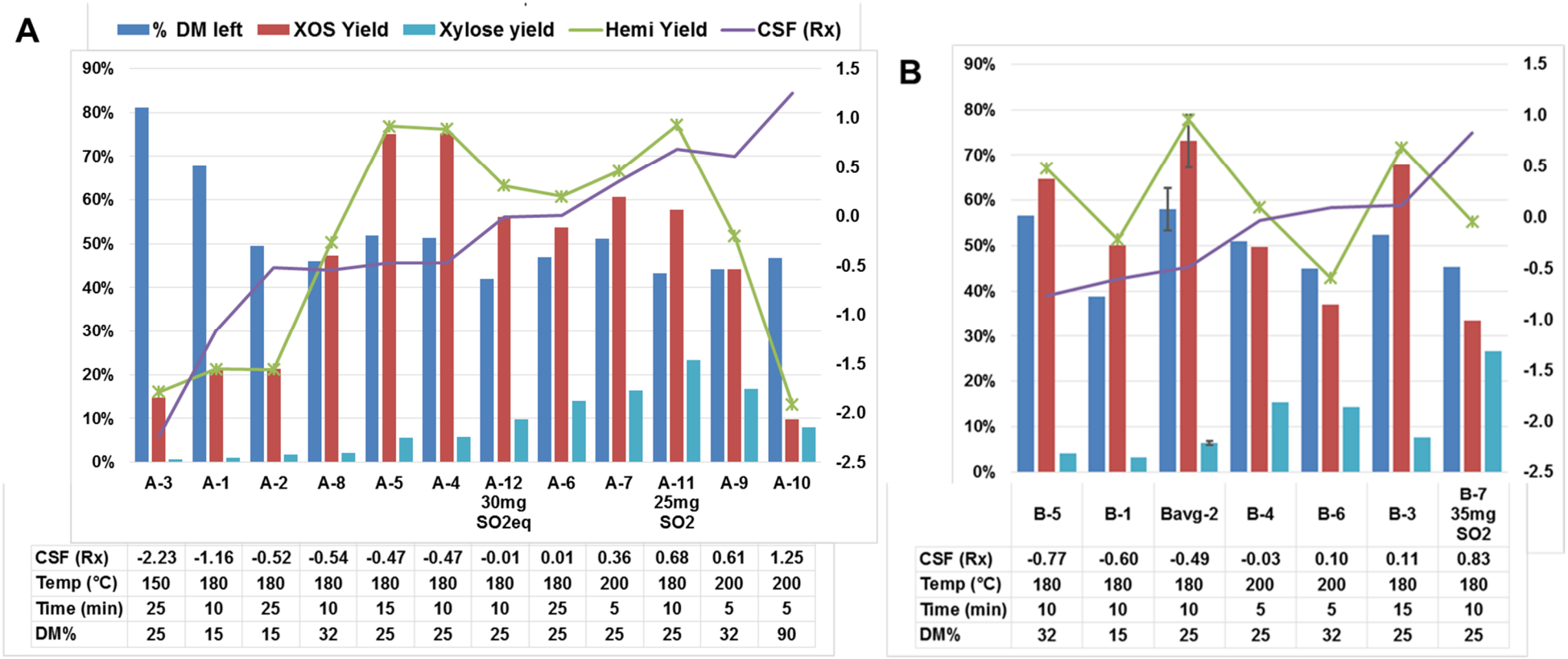
5.3.2.1 XOS production scale-up in SE by using optimised bench scale LHW process conditions and comparison of CSF

Bench scale LHW optimisations in stirred batch reactors provide good starting points for process conditions to scale up in SE HTT. Firstly, from the LHW HTT process conditions (Chapter 4) for the highest XOS yield (78.0%) obtained using WBSG-15% (180 ºC and 15 min) did provide process conditions resulting in similar high XOS yields (75.1%) in SE HTT (run A-5) at the same process condition using 25% dry matter (Figure 5-1 A). However, by a comparison of all the SE runs using CSF it is clearly seen, as shown in Figure 5-1 A and B, the preferred process conditions of 10 min at 180 °C selected from reported optimisations in LHW HTT (11% dry matter) obtained the highest XOS yields (>73%) for both types of BSGs (25% dry matter) at a CSF of *ca* -0.48 (run A-4 and run B-2). XOS yields increased with increasing CSF from -2.5 to around -0.48 to achieve the highest yields. From a CSF of around 0, a further increase resulted in significantly increased xylose formation from increased XOS depolymerisation. Both BSG's show a turning point at CSF of *ca* -0.48 for fractionating a large XOS fraction from BSG (>73%) with minimal xylose formed (Figure 5-1 A and B). Moreover, the preferred process conditions of 10 min at 180 °C selected, provided the highest ArOS yield (Figure 5-2 A). An increased time to 15 min from 10 min, resulted in a reduction in ArOS yields (Figure 5-2 A) and degradation by-product formation increased significantly from 0.81 and 1.10 g/100g dm to 1.48 and 1.68 g/100g dm, for WBSG and PBSG, respectively (Figure 5-2 B). Therefore, both the reported (11% dry matter) optimal process conditions for maximum XOS production (XOS +ArOS) from bench-scale multivariate optimised LHW HTT of BSG (Gomez *et al.*, 2015) and process conditions found with highest XOS yield in LHW HTT using

15% dry matter content (Chapter 4) did provide process conditions for use in scale-up in SE HTT. This is in agreement with literature that demonstrated the use of bench scale HTT optimisations for total xylan yield from cornstover in HTT scale-up (Lischeske *et al.*, 2016). This study showed that less costly smaller bench scale HTT optimisations, even from literature, can be useful to assist process condition in scale up to larger HTT systems like SE.

5.3.2.2 Effect of variations in chemical composition of BSG on XOS production in SE HTT

The XOS production scale-up in SE was not significantly affected by small differences in chemical compositions. Applying a selected/preferred set of conditions to different types of BSG, resulted in yields and XOS product qualities that were comparable. The highest XOS yields for both Weiss (WBSG) and Malt based (PBSG) BSG's were obtained at the same process condition of 180 ºC and 10 min using 25% dry matter (Figure 5-1 A and B). Differences in composition between WBSG and PBSG only led to significant differences in yields of degradation by-products, acetic (higher for PBSG) and formic acid (lower PBSG). Moreover, the significantly higher starch content in WBSG, did not have a significant effect on HMF yields from WBSG compared to PBSG (Figure 5-2 B). These comparable HTT results can also be found between various reported LHW HTT optimisation studies for BSG's where similar process conditions obtained comparable (*circa* 60%) high XOS yields (Carvalheiro *et al.*, 2004; López-Linares *et al.*, 2019). Additionally, comparing the XOS and xylose yields across reported LHW HTT from BSG (Carvalheiro *et al.*, 2004) with SE HTT show similar trends for xylose and XOS formation when using CSF that incorporates the resulting pH obtained from the HTT (Figure 5-3). The xylose yield increased significantly from a CSF of *ca* -0.5, at the conditions of highest XOS yield, for both WBSG, run A-4, and PBSG, run B-2 and the optimised process condition in LHW HTT reported (Figure 5-3). Therefore as this study showed, optimal process conditions from bench scale HTT studies in literature that use other BSG's, with small differences in chemical compositions, can be useful to find preferred process conditions from scale up.



	A-3	A-1	A-2	A-8	A-5	A-4	A-12 30mg SO2eq	A-6	A-7	A-11 25mg SO2	A-9	A-10
CSF (Rx)	-2.23	-1.16	-0.52	-0.54	-0.47	-0.47	-0.01	0.01	0.36	0.68	0.61	1.25
Temp (°C)	150	180	180	180	180	180	180	180	200	180	200	200
Time (min)	25	10	25	10	15	10	10	25	5	10	5	5
DM%	25	15	15	32	25	25	25	25	25	25	32	90

	B-5	B-1	Bavg-2	B-4	B-6	B-3	B-7 35mg SO2
CSF (Rx)	-0.77	-0.60	-0.49	-0.03	0.10	0.11	0.83
Temp (°C)	180	180	180	200	200	180	180
Time (min)	10	10	10	5	5	15	10
DM%	32	15	25	25	32	25	25

Figure 5-1 A comparison of steam explosion results: (A) WBSG and (B) PBSG using combined severity factor (CSF): [■] Insoluble solid residue, [■] XOS yield, [■] Xylose yield, [✳] Hemicellulose yield, [—] CSF (Right axes) (Experimental 95% confidence error bars of triplicate runs B-2)

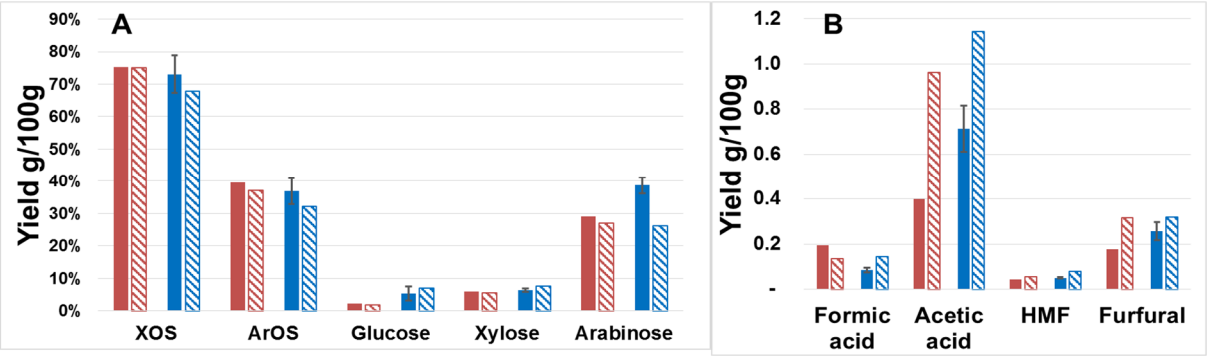


Figure 5-2 SE treatment results for 180 °C and 25% dm: (A) Product yield and (B) inhibitors yield (g/100 g dry BSG); (10/15 min [■/▨] WBSG and 10/15 min [■/▨] PBSG)

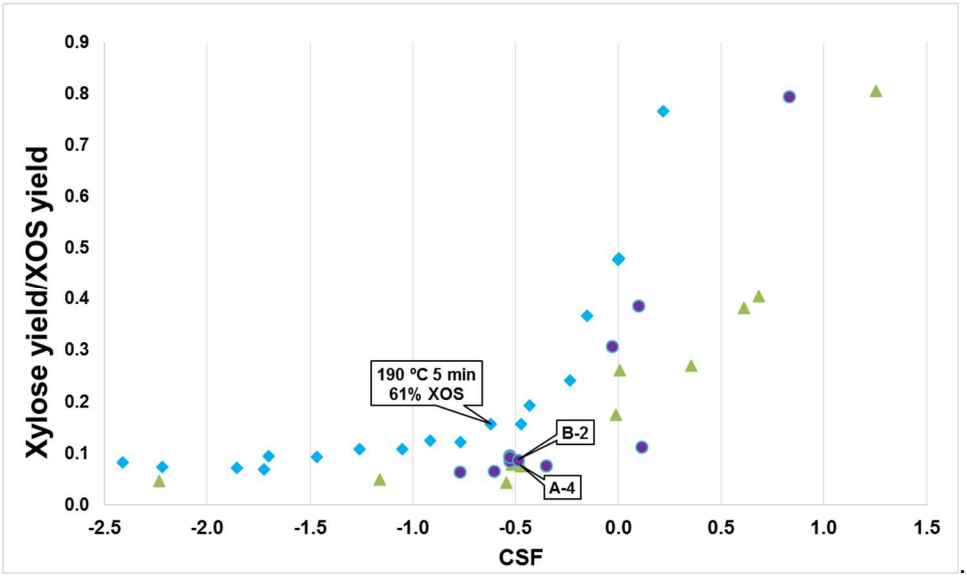


Figure 5-3 Combined severity factor for steam explosion and LHW HTT comparison: Steam explosion of [▲] WBSG and [●] PBSG; and reported HTT of BSG, [◆] 11% dm autocatalytic LHW (Carvalheiro *et al.*, 2004) (Highest XOS yield runs indicated)

5.3.2.3 Increasing solids loadings with screw press dewatering for higher XOS yields in SE XOS production

Higher dry matter concentrations (>11% dm) in HTT can lead to higher product yields. Based on literature (Modenbach and Nokes, 2012), it is to be expected that higher dm in HTT will have negative effects on product yields, while increasing degradation by-products formation. Yet, contrary to that expected, using 25% dm for both WBSG (run A-4) and PBSG (run B-2) in SE HTT increased the XOS yield >73% at 180 ºC and 10 min compared to only 21.1% and 50.0% using 15% dm for WBSG and PBSG respectively (Figure 5-4 A1). The higher XOS yields from 25% dm BSG's were obtained even with slightly higher xylan contents in the 25% dm BSGs' compared to the 15% dm (Table 5-2). Nevertheless, these SE HTT findings of increasing XOS yields from 15% to 25% dry matter BSG are in agreement with studies of barley husks in SE that reported increasing dry matter from 20 to 30% resulted in increased

xylan recovery (Roos *et al.*, 2009) and in unstirred autocatalytic LHW of bagasse at 170 °C, where XOS yield increased from 34% to 43% with increased dry matter content of 10% to 25% at the same treatment time (Vallejos *et al.*, 2012). The increase in XOS yield in SE HTT of screw pressed BSG observed in this study can be as a result of a combination of the effect of reduced moisture content and buffering capacity decreasing the resultant pH in the SE HTT (Mosier, Ladisch and Ladisch, 2002). Additionally, the screw press dewatering increased BSG bed porosity (reduced bulk density Table 5-2), increasing steam penetration during heating in a screw pressed dried BSG compared to the raw BSG, creating faster and more uniform heating patterns (Brownell and Saddler, 1986). Since temperature is a key factor in HTT, efficient exposure at the required temperature permits improved HTT of biomass since improved steam penetration will allow better temperature control, including more rapid heat-up and more accurate control at the set point (Cullis, Saddler and Mansfield, 2004; Sui and Chen, 2015).Therefore, the increasing yields obtained with increasing solids loading in SE HTT in this study support the inclusion of dry matter content or moisture as an additional optimisation parameter with time and temperature in HTT.

5.3.2.4 Screw press dewatering for optimising product yields in SE HTT

The use of higher dry matter concentrations (>11% dm) in HTT can have significant process advantages if the negative effects of increased degradation by-products formation on process yields can be mitigated with effective optimisation strategies. Screw press dewatering of BSG can enable optimisation of XOS production in SE HTT by maximising the XOS yield as seen from results at the preferred process condition of 180 ºC and 10 min for both BSG's (Figure 5-4 A1). XOS yields of WBSG-15% (21.1%) increased to 75.3% for WBSG-25% and then decreased to 48.3% for WBSG-32% (Figure 5-4 A1). Similarly for PBSG the XOS yield increased at 180 °C and 10 min, from 50.0%, for PBSG-15%, to 73.1% (highest XOS yield) for PBSG-25% and this decreased again to 64.8% with PBSG-32%. Degradation by-products formation support this optimisation, as the acetic and formic acid formation increased with dry mater content of BSG between 15% to 25% dry matter and decreased between 25% to 32% dry matter, for both types of BSG (Figure 5-4 A2). However at 200 ºC, the further increase in dry matter content to 32% further increased degradation byproduct formation from 1.54 to 1.57 and from 2.06 to 2.46 g/100g dm for WBSG and PBSG respectively (Figure 5-4 B2), contributing in reduced XOS yields. Moreover, at 200 °C, a significant negative effect of increased dry matter content on XOS yield is evident from the treatment of the air dried 90% dry matter WBSG (original 25% dry matter) where the SE yield of XOS dropped from 60.6%, with 25% dry matter WBSG. to 9.8%, with 90% dry matter (air dried WBSG-90%). From the results it is clear moisture content significantly affects product and degradation by-product

formation in SE HTT and needs to be optimised in the HTT in combination with time and temperature.

5.3.2.5 Overall XOS production process intensification by screw press dewatering

Using higher dry matter concentrations (>15%) of BSG through screw press dewatering in a SE HTT process can provide significant HTT process improvements for the intensification of the production of XOS. This is achieved through: (I) achieving similarly high (>73%) XOS yields in SE HTT compared to reported nonisothermal (200 ºC) optimised LHW studies (Gomez *et al.*, 2015) and increasing (>10%) XOS yields from reported isothermal (190 ºC and 5 min) optimised LHW studies (Carvalheiro *et al.*, 2004; López-Linares *et al.*, 2019); (ii) together with a reduction in the required process water in HTT of more than 60% by processing 25% dry matter BSG in SE compared to 9-11% dry matter in reported LHW studies; (iii) additionally, reduced HTT process temperatures by up to 20 ºC from 200 ºC were achieved compared to the reported LHW HTT optimisations (Carvalheiro *et al.*, 2004; Gomez *et al.*, 2015; López-Linares *et al.*, 2019) while maintaining or increasing product yields.(iv) A further reduction in degradation by-product formation compared to stirred batch work (Chapter 4) as was shown in the application of appropriate high solids HTT processing of BSG in SE at 25% dm (75.3% XOS yield) that resulted in degradation by-product formation of only 0.81 g/100 g dm compared to 1.50 g/100 g dm with 15% dm (Chapter 4 - 78.0% XOS yield) and 1.89 g/100 g dm reported at optimised conditions (190 ºC and 5 min) in stirred batch reactors (Carvalheiro *et al.*, 2004), conducted with 11% dm (61% XOS yield). (v) Moreover, overall product recovery increased. Compared to the reported SE HTT optimisation by Rojas Pérez, (2018) on BSG for maximum (47.0%) total xylan yield (XOS + xylose), this SE HTT study achieved significantly higher (>30%) total xylan yields at the preferred process conditions for both WBSG and PBSG.

Thus, the combination of effects of higher XOS product yield and concentrations, lower water usage, lower processing temperature requirements and lower degradation by-product formation achieved in this study could lower process energy and reduced equipment requirements that combined contributed to production costs that can enabled BSG valorisation at small scale.

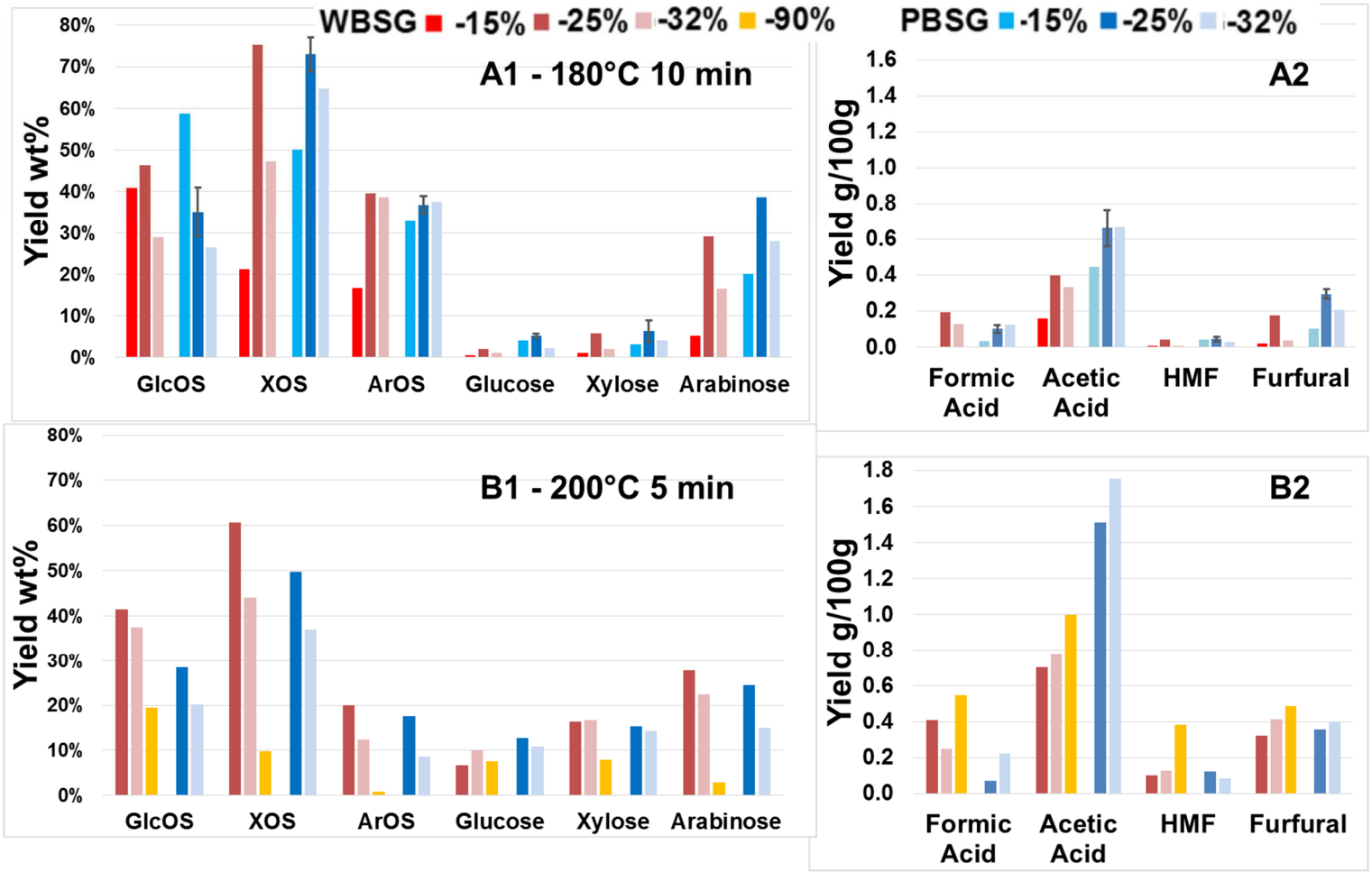


Figure 5-4 SE treatment results for WBSG and PBSG: (A). 180 °C and 10 min; (B). at 200 °C and 5 min; (1) Product yield and (2) inhibitors yield (g/100 g dry BSG) ([■] 15% dm, [■] 25% dm, [■] 32% dm and [■] 90% dm WBSG and [■] 15% dm, [■] 25% dm and [■] 32% dm PBSG)

5.3.2.6 XOS depolymerisation to short chain XOS with SO_2 addition and high severity SE HTT

XOS is reported to be a functional food ingredient, an antioxidant and more importantly, short chain XOS oligomers with DP<7, are reported to be prebiotic with bifidogenic activity of which the xylobiose (X2) and xylotriose (X3) obtained from BSG showed the most activity (Gomez *et al.*, 2015). However, BSG SE results showed that at conditions for the highest XOS yield (180 °C at 10 min with 25% dm), the XOS obtained contained low combined fractions (<10%) of these two short chain XOS from both types of BSG's (Figure 5-5). As an alternative to increase this fraction, by increasing the CSF at the SE condition for the highest XOS yield, SO_2 catalyst additions were tested. Direct SO_2 dosing by solution in run A-11 for WBSG-25% (Figure 5-1 A), produced a significantly higher CSF (0.68) compared to the $K_2S_2O_5$ addition (-0.01) run A-12. In run A-11 for WBSG-25%, direct SO_2 solution dosing, 25 mg SO_2/g dry feedstock increased the combined X2 and X3 fraction to 16.5 % (Figure 5-5) and obtained the highest yield of hemicellulose (77.1%) and highest xylose yield (23.3%) from WBSG (Figure 5-1 A). The 35 mg SO_2/g dry feedstock dosing in SE of PBSG-25%, run B-7, increased the CSF to 0.83 (from -0.49 without SO_2) which increased the combined X2 and X3 fraction to 25.3% in run B-7 for PBSG (Figure 5-5). However, the SO_2 catalyst addition reduced XOS yield from 73.1% to 33.6% as a result of increased severity from the SO_2 catalyst. Yet, without additional chemical use, by only increasing the SE HTT process conditions to 200 °C and 5 min (25% dm), a similar increase of the combined fraction of X2 and X3 (27.0%) with an overall better XOS yield (49.7%) was achieved. Additionally, the screw pressed PBSG-32% obtained the highest fraction of 31.3% at 200 °C and 5 min that also show the increased severity effect from the screw press dewatering step. These findings are significant as a possible single production step of XOS with SE that could provide a large portion of prebiotic X2 and X3, either at high severity (200 °C and 5 min) or aided with SO_2 catalyst.

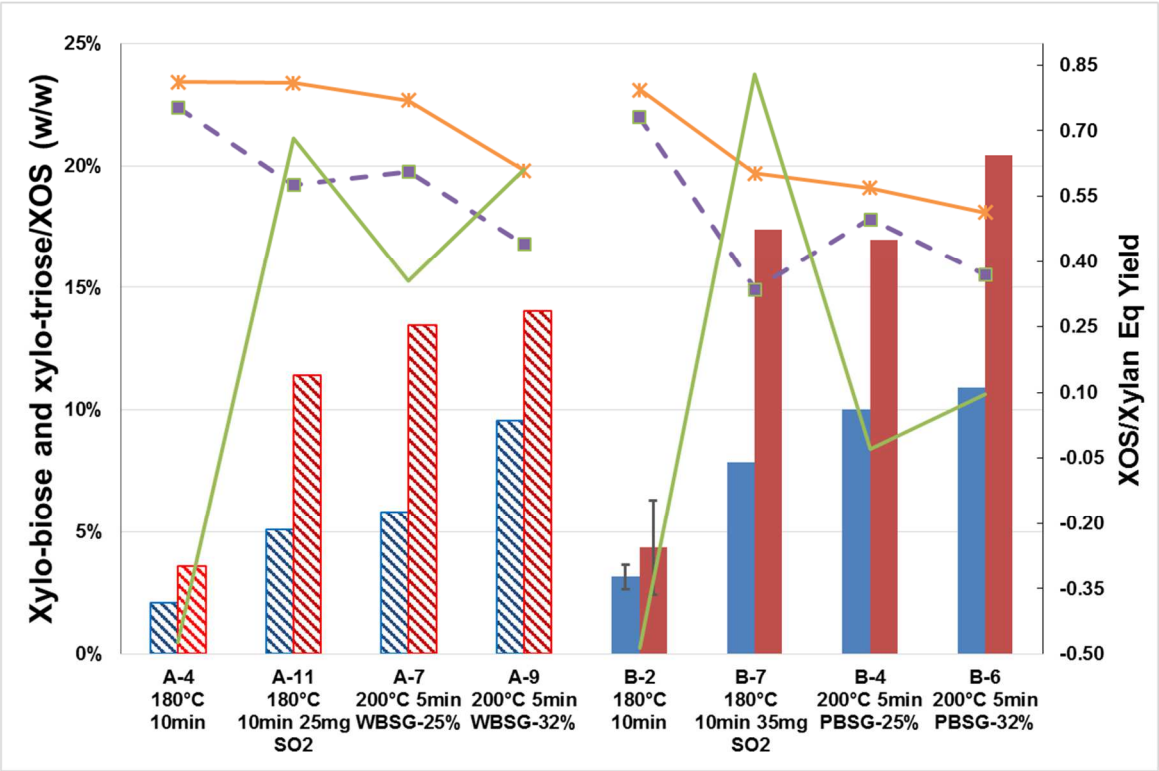


Figure 5-5 XOS composition: [/] Xylobiose (X2) and [/] xylotriose (X3) from steam explosion of 25 and 32% dm; (Left axis; Yield in XOS, Dashed //; for WBSG, and Solid [/] for PBSG, Right axis; [] CSF, [] XOS Yield, and [] Xylan Eq Yield)

5.3.3 Effect of screw press dewatering on HTT, acidification and the resulting hydrolysate pH

Screw press dewatering of BSG in SE was shown to have a significant effect on the HTT process, affecting the hemicellulose xylan solubilisation, XOS yields, resulting pH and CSF (Table 5-3). Similar to the increased xylan solubilisation obtained in SE HTT at 180 ºC and 10 min by screw press dewatering BSG from 15% and 25% dm, screw press dewatering also accelerated starch hydrolysis in SE HTT with reducing the residual starch in the remaining insoluble solid from WBSG-15%, from 8.1% to 0.8% with the pressed WBSG-25% and from 2.2% to 0.1% with the PBSG-25% at the same process conditions (Appendix Figure 5A-3).Yet in HTT, the rate of solubilisation and depolymerisation of xylan and glucan polysaccharides is related proportionally to the H+ concentration in the hydrolysate (Mosier, Ladisch and Ladisch, 2002). In autocatalytic HTT the effect of water as a weak acid is the main catalyst affecting the H+ concentration and, during HTT, acidification can be accelerated through the release of acetic, furanic, uronic and phenolic acids (Ibbett *et al.*, 2011; Negahdar, Delidovich and Palkovits, 2016). External catalysts such as H_2SO_4 or SO_2 can be added to increase the H+ concentrations in autocatalytic HTT such with ELA (extremely low acid) dosing (Chapter 4).

5.3.3.1 Screw press dewatering modification of polysaccharide solubilisation and depolymerisation in SE HTT

The autocatalytic HTT solubilisation and depolymerisation of hemicellulose xylan and starch (glucan) in the steam explosion and LHW are comparable. Xylan and glucan depolymerisation yields change proportionally, even considering the small differences in the compositions of WBSG and PBSG, and modifications with the screw press dewatering (Figure 5-6). However the addition of SO_2 catalyst in SE (run A-12 and B-7) and ELA LHW (Chapter 4) changed the xylan and glucan depolymerisation significantly compared to autocatalytic HTT with catalyst additions resulting in significantly more glucose formation relative to xylose. Autocatalytic depolymerisation even with the highest CSF 1.25, in run A-10, from the 90% air dried WBSG (original WBSG-25%) obtained xylan and glucan depolymerisation results similar to other more dilute and less severe autocatalytic SE runs of WBSG and PBSG (Figure 5-6). This is consistent with literature that showed, for straw, that mainly HTT reactions are activated with temperatures of up to 200 ºC, even at high dry matter content (<80%) (Ibbett *et al.*, 2011). This suggest that the effect of compositional modifications with screw press dewatering on BSG could affect rates of polysaccharide solubilisation and depolymerisation similar to moisture reductions. Screw press dewatering is effective to modify the rate of polysaccharide solubilisation and depolymerisation in autocatalytic HTT.

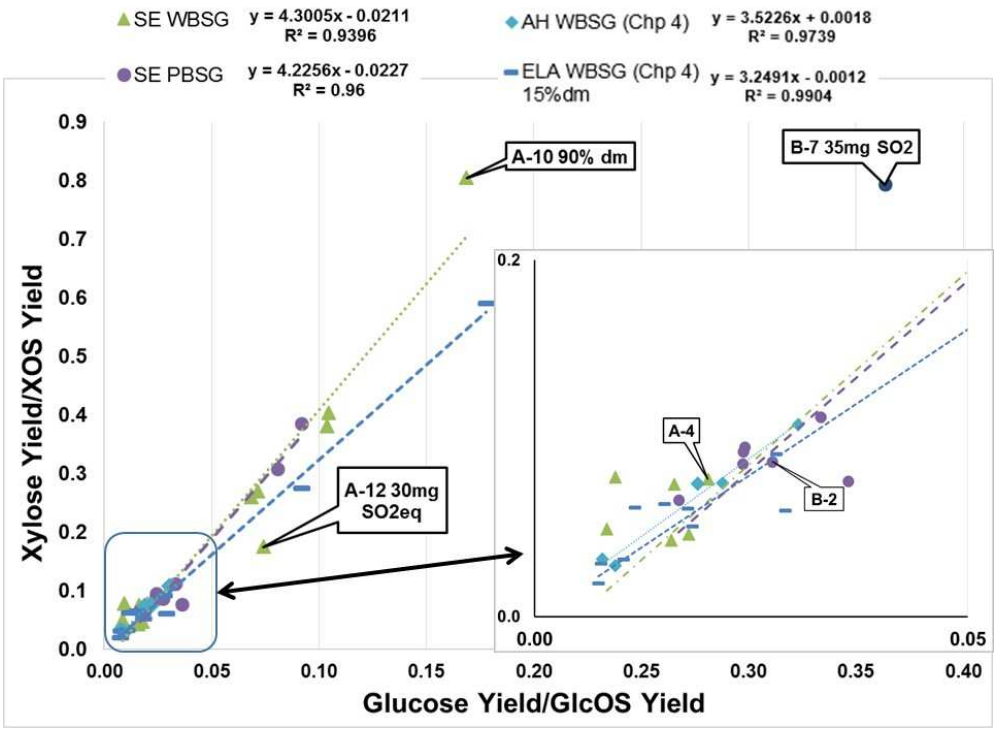


Figure 5-6 Monomeric xylose and glucose formation relative to oligomers in HHT: Steam explosion of [▲] WBSG and [●] PBSG; and WBSG in liquid hot water, [◆] autocatalytic and [–] Extremly low acid (ELA) catalysed (Chapter 4)

5.3.3.2 Screw press dewatering and initial dry matter content to adjust H+ concentration, pH and CSF in autocatalytic SE HTT

As expected, the screw press dewatering and temperature played a significant role in SE HTT hydrolysate acidification from both types of BSG's. Two distinct trends were found when looking at the effects of screw press dewatering on hydrolysate acidification (mol H+ per gram dry BSG) in SE HTT from WBSG and PBSG (Figure 5-7 A and B). Firstly at 180 ºC, increasing screw pressed dry matter content from 25% to 32% resulted in reduced acidification of between 35-57% in autocatalytic SE HTT for both BSG's (Figure 5-7 A and B) when compared to 25% dm and accounting for the resulting dilution effect of SE in the hydrolysates (Appendix Figure 5A-4). This reduction in acidification is also shown in ratio of R[H+] 0.43 for PBSG 32%/25% and R[H+] 0.66 for WBSG 32%/25% (Table 5-3 (a)), even though with 32% dm WBSG the hydrolysate H+ concentration increased slightly (lowered pH). This reduced acidification at 32% dm for both WBSG and PBSG can explain the lower XOS yields from 60.6 to 44.1% obtained with the 32% dry matter BSG's in this study (Table 5-3 (a)), compared to 25% dm. This is in agreement with reported findings where rate of xylan solubilisation reduced by 66% with a doubling in dry matter content in HTT (Morinelly *et al.*, 2009).

Secondly, at 200 ºC the same dewatering increased hydrolysate acidification in HTT (Figure 5-7 A and B). Increased screw pressed dry matter content from 25 to 32% dry matter leads to increased acidification compared to 180 ºC and 10 min for both BSG's with R[H+] for WBSG-32%25% at 200ºC relative to 180 ºC is 1.75 (1.15/0.66) and for PBSG 32%25% is 2.93 (1.26/0.43) (Table 5-3 (a)). This significant increased acidification at 200 ºC compared to 180 ºC could be as a result of more exothermic hydrothermal carbonisation reactions activated from temperatures around 200 ºC accelerating HTT acidification (Ibbett *et al.*, 2011). This increasing acidification at 200 ºC with dry matter was also found to be similar for screw press dewatering and moisture reduction.

The screw press dewatering of BSG could be considered as a moisture reduction step, since the acidification (mol H+ per gram dry BSG) in the hydrolysate from screw pressed BSG in autocatalytic SE HTT was not significantly different from that obtained by moisture reduction alone. A reduced water content or increasing the dry matter content from 25% to 90% dm (air dried) process at the same conditions (5 min and 200 ºC) in the SE HTT resulted in a significant reduction in hydrolysate pH (from pH 3.33 in run A-7 to pH 2.43 in run A-10) (Table 5-3 (a)), which increased severity in SE HTT with increased CSF (from 0.36 (A-7) to 1.25 (A-10)). However, this increased acidification at 200 ºC and 5 min for WBSG-90% (air dried) was proportional to the increased acidification with WBSG-32% (screw pressed) by the reduction in water (Figure 5-7 A). Therefore, the H+ concentration obtained and H+ released during SE HTT of screw pressed BSG seems relate to the reduced water content and the fractionation

of components including starch, protein and buffering components did not show significant effects. The finding is in agreement with results from autocatalytic LHW HTT in stirred batch reactors that found comparable acidification (H+ per gram dry BSG) for raw and screw pressed WBSG at 150 °C and 180 °C despite differences in composition (Figure 5-7 A).

5.3.3.3 Screw press dewatering in pilot-scale autocatalytic SE HTT compared to bench-scale autocatalytic LHW

The stirred batch was more suitable for lower (up to 15% dm) solids loadings autocatalytic HTT of BSG compared to unstirred SE since the acidification (mol H+ per gram dry BSG) was almost constant in stirred reactors, while SE HTT was better suitable for higher (*circa* 25% dm) solids loadings (Figure 5-7 A). SE HTT of WBSG-15% at 180 ºC for 10 min (run A-1) achieved only 29% of the acidification of 5 min autocatalytic LHW HTT in a stirred batch reactor (Table 5-3 (b)). However, the screw press dewatering of WBSG-15% to WBSG-25% improved acidification in the pilot-scale SE HTT that was more beneficial to XOS production in comparison with the bench scale LHW HTT since this increased acidification with dewatering increased XOS yields in SE (Table 5-3 (b)). SE HTT screw pressed WBSG-25% at the preferred process conditions (run A-4), achieved 73% acidification and similar CSF conditions than LHW (run AH-A3) of raw WBSG-15% in the stirred batch Parr reactor (Table 5-3 (b)), treatments that both resulted in maximum XOS yields (78.0% and 75.3%). This observation could be attributed to the screw press process creating large void fractions, increasing the bed porosity of WBSG-25%, resulting in increased steam permeability, more efficient heating with a reduction in moisture content, summing up to a significantly improved autocatalytic effect in the SE HTT process (Brownell and Saddler 1986; Cullis, Saddler and Mansfield, 2004; Sui and Chen, 2015). In summary, a high dry matter content (WBSG-25%) in a SE HTT is as effective in the production of XOS as a low dry matter content (WBSG-15%) in a LHW HTT process.

Table 5-3 Effect of dry matter content achieved by screw press dewatering on the resulting pH and H+/g dry BSG in autocatalytic HTT: (a) Comparison of SE treatments for different screw press dewatered BSGs, (b) Comparison between SE and LHW treatments using similar starting BSG

(a) Steam explosion

BSG type	WBSG					PBSG		
Temperature (°C)	180	180	180	200	200	180	180	200
Time (min)	10	25	10	5	5	10	10	5
Set A	A-1	A-2	A-4	A-7	A-7	B-1	B-2	B-4
dm%	15	15	25	25	25	15	25	25
pH	4.55	4.29	4.02	3.33	3.33	3.99	3.92	3.71
CSF	-1.16	-0.52	-0.47	0.36	0.36	-0.60	-0.49	-0.03
XOS Yield%	21.1	21.4	75.3	60.6	60.6	50.0	73.1	49.7
Set B- Run	A-4	A-6	A-8	A-9	A-10	B-2	B-5	B-6
dm%	25	25	32	32	90	25	32	32
pH	4.02	3.76	3.93	3.07	2.43	3.92	4.16	3.59
CSF	-0.47	0.36	-0.54	0.61	1.25	-0.49	-0.77	0.10
XOS Yield%	75.3	53.7	47.3	44.1	9.8	73.1	64.8	37.0
Resulting ratio (R[H+]) mol H+ in B / in A	2.17	1.43	0.66	1.15	1.54	0.65	0.43	1.26

(b) Steam explosion vs LHW

BSG type	WBSG		
Temperature (°C)	180	180	180
	Autocatalytic LHW Parr[b]		
Set A	AHA2	AHA3	AHB2
time (min)	5	15	5
dm%	15	15	25
pH	3.84	4.02	3.54
CSF	-0.30	-0.40	-0.06
XOS Yield%	65.0	78.0	65.3
	Steam explosion		
Set B	A-1	A-4	A-4
time (min)	10	10	10
dm%	15	25	25
pH	4.55	4.02	4.02
CSF	-1.16	-0.47	-0.47
XOS Yield%	21.1	75.3	75.3
Resulting ratio (R[H+]) mol H+ in B / in A	0.29	0.73	0.44

R[H+]) basis of gram dry BSG; [b] Own results to be published (Chapter 4)

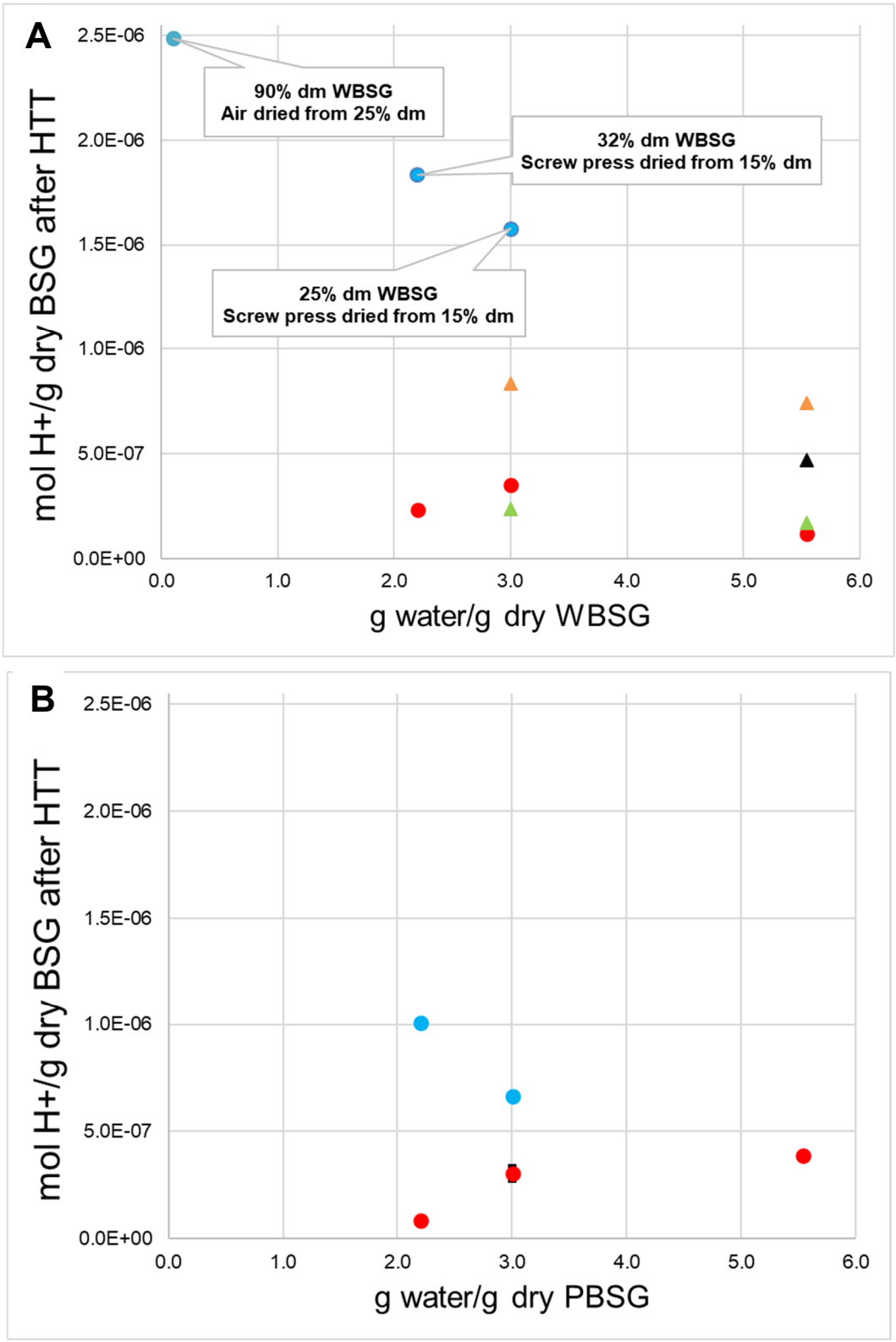


Figure 5-7 The effect of screw press dewatering on acidification in HTT of (A) WBSG and (B) PBSG: Steam explosion at [●] 180 ºC for 10 min and [●] 200 ºC for 5 min and; Stirred batch reactor (Chapter 4) at [▲] 150 ºC for 10 min and [▲] 180 ºC for 5 min and [▲] 15 min.

5.3.4 Effect of screw press dewatering on SE hydrolysate purity

Hydrolysed starch, proteins and other soluble by-products including degradation products formed from components in SE of BSG, negatively affect the purity of the XOS produced. Purification of the hydrolysate for XOS is a large cost component in XOS production and improving the purity needs to be considered (Amorim, Silvério and Rodrigues, 2019).

5.3.4.1 Screw press dewatering for highest fraction of XOS in TDS of hydrolysate

Higher oligosaccharide fractions in total dissolve solids (TDS) were obtained from SE HTT with short residence times, lower temperatures and high dry matter content screw pressed BSGs (Table 5-4 A and B). The total oligosaccharide (OS) fraction (XOS and ArOS) increased in the TDS of the SE hydrolysates from screw pressed BSG's through removal of water soluble materials and impurities which include minerals, starch and proteins. Results show a highest OS/TDS of 51.9% and 42.2% was obtained for WBSG (32% dm) and PBSG (32% dm) respectively, both at 180 °C and 10 min (the preferred HTT process conditions) (Table 5-4 A and B). To improve on the process minimising purification cost, through increasing the purity of XOS, ideally the screw press could be used to dry the BSG to the minimum moisture content of *circa* 40% dry matter and then the water content can be readjusted to the dry matter content required (25% dm) for optimum yield of the XOS. Additionally, such increased screw press intensity can result in higher recovery of protein in the press water as a valuable by-product.

The addition of SO_2 catalysts at the process conditions for the highest XOS yield resulted in increased soluble components in the hydrolysate that reduced. OS/TDS from 37.1% for WBSG (run A-4) without catalyst addition to 23.9 to 25.9% (Table 5-4 A). Therefore, the catalyst addition at the tested conditions did not improve XOS purity or yield in autocatalytic SE process mainly since it increased depolymerisation.

Table 5-4 (A) Steam explosion results for WBSG

Run	Dry Mass%	Temp (°C)	Time (Min)	XOS Yield	ArOS Yield	Hemi Yield	%XOS	%ArOS	Inhibitor (g/100g)	OS[e]/ TDS	Final dm%	CSF	Mass Balance
A-1	15	180	10	21.1%	16.8%	21.2%	95.3%	75.8%	0.18	21.0%	13.7%	-1.16	84.5%
A-2	15	180	25	21.4%	14.0%	21.1%	92.9%	68.4%	0.23	15.5%	8.6%	-0.52	65.9%
A-3	25	150	25	14.7%	11.7%	16.0%	95.6%	60.8%	0.24	18.6%	9.0%	-2.23	79.6%
A-4	25	180	10	75.3%	39.6%	76.2%	92.8%	57.7%	0.81	37.1%	20.2%	-0.47	88.6%
A-5	25	180	15	75.1%	37.0%	76.9%	93.0%	58.1%	1.46	36.9%	22.6%	-0.47	94.5%
A-6	25	180	25	53.7%	15.6%	60.8%	78.9%	41.7%	1.78	27.1%	18.2%	0.36	82.2%
A-7	25	200	5	60.6%	20.0%	66.6%	78.8%	41.9%	1.54	29.2%	27.7%	0.36	86.5%
A-8	32	180	10	47.3%	38.6%	50.3%	95.9%	70.1%	0.51	51.9%	35.4%	-0.54	66.2%
A-9	32	200	5	44.1%	12.4%	51.7%	72.4%	35.6%	1.57	24.8%	32.3%	0.61	75.7%
A-10[a]	90	200	5	9.8%	0.9%	13.2%	55.4%	23.0%	2.41	7.5%	59.9%	1.25	67.2%
A-11[b] 25mg SO_2	25	180	10	57.7%	22.4%	77.1%	71.2%	31.2%	0.96	25.9%	17.8%	0.68	82.6%
A-12[c] 30mg SO_2eq	25	180	10	56.0%	27.4%	63.3%	85.1%	44.7%	1.13	23.9%	19.0%	-0.01	84.5%

a Pressed and air dried to 10% moisture; b/e Added as 4.5% SO_2 solution; c Added as $K_2S_2O_7$ solid ; d triplicate runs e OS Oligomers XOS and ArOS yield combined;

Table 5-4 (B) Steam explosion results for PBSG

Run	Dry Mass%	Temp (°C)	Time (Min)	XOS Yield	ArOS Yield	Hemi Yield	%XOS	%ArOS	Inhibitor (g/100g)	OS[e]/ TDS	Final dm%	CSF	Mass Balance
B-1	15	180	10	50.0%	32.8%	51.3%	93.9%	62.1%	0.62	29.4%	11.9%	-0.60	70.7%
B-2[d]	25	180	10	73.1%	36.9%	77.8%	91.9%	49.3%	1.10	40.6%	19.9%	-0.49	94.6%
B-3	25	180	15	67.9%	32.0%	71.7%	89.9%	55.2%	1.68	41.5%	20.6%	0.11	86.8%
B-4	25	200	5	49.7%	17.5%	58.4%	76.2%	44.0%	2.06	21.4%	18.6%	-0.03	81.3%
B-5	32	180	10	64.8%	37.5%	67.0%	94.0%	57.2%	1.03	42.2%	24.9%	-0.77	89.0%
B-6	32	200	5	37.0%	8.7%	42.9%	72.2%	36.7%	2.46	24.6%	19.5%	0.10	73.5%
B-7[e] 35mg SO_2	25	180	10	33.6%	12.2%	55.3%	55.7%	25.8%	2.52	21.1%	15.6%	0.83	76.9%

a Pressed and air dried to 10% moisture; b/e Added as 4.5% SO_2 solution; c Added as $K_2S_2O_7$ solid ; d triplicate runs e OS Oligomers XOS and ArOS yield combined;

5.3.4.2 Screw press dewatering for higher protein recovery

Protein recovered from BSG can be a valuable by-product in a valorisation concept of BSG with novel applications in food products (Celus, Brijs and Delcour, 2009; Connolly *et al.*, 2017; Cian *et al.*, 2018). Removing a fraction of the BSG protein with the screw press dewatering before the SE, improved the overall recovery of proteins in the fractionation process, together with avoiding undesired degradation in SE (Rommi *et al.*, 2018) and improved the OS/TDS in the SE hydrolysate. The screw press protein fractions separated, for both PBSG and WBSG, show selectivity to the non-essential amino acid proline and glutamine (Table 5-5). The overall protein balance of the process for SE conditions of highest XOS yield (run B-2), using the crude protein contents for the starting PBSG and SE residue (Appendix Table 5A-1), show up to 73.6% of PBSG protein can be recovered as insoluble residue (press liquid and SE residue). The SE of PBSG-25%, aided with addition of SO_2 catalyst, removed more proteins compared to autocatalytic SE. Low severity SE HTT had a better recovery of PBSG proteins in the remaining solids. SE process conditions at 200 °C and 5 min removed 32.3% from the overall crude protein of raw PBSG-15% compared to 26.4% at 180 °C and 10 min. While lower severity is preferred, SE of BSG can recover protein (rich in essential amino acids) in the remaining solid fraction, as a valorisation step (Tucker *et al.*, 2004). However, the SE residue composition at the near optimal conditions for PBSG-25% (run B-2) results show 81.7% of the nitrogen of PBSG-25% removed in SE was accounted for in the hydrolysate. The hydrolysate for run B-2, showed 15.3% crude nitrogen in TDS, of which 66.2% was accounted for as amino acids (Table 5-5). The solubilised crude nitrogen made up 53.4% of other components non-determined, which is similar to reported autocatalytic LHW optimised conditions for XOS production (Gomez *et al.*, 2015).

Protein content determination by amino acid and crude protein values for the same fractions varied (Table 5-5). The total amino acid values for screw press insoluble solids were slightly higher compared to the crude protein which underestimated the protein fraction in the press

fractions (Table 5-5). On the contrary, the crude protein from the SE residues were consistently higher than the total amino acids even though tryptophan and cysteine amino acids were not quantified (reportedly <2% combined content in total BSG amino acids) as shown in Table 5-5. The solid residue from SE run B-4 at 200 °C showed a crude protein of 19.6% while the amino acid total was 14.4%. That shows that almost 20% of the nitrogen was degraded in the insoluble residue in the SE HTT (Table 5-5). Acid insoluble (AI) fractions from WBSG and PBSG showed an increase from 22.4% to 24.5% for BSG and between 32.3 to 34.5% in residues from SE treatment (Appendix Table 5A-2). The AI fraction from SE run B-2 at 180 °C for highest XOS yield contained 39.6% crude protein, and the addition of SO_2 in SE reduced the crude protein fraction in the acid insoluble fraction to 35.7%. Reports have shown that BSG proteins degrade in SE by aggregation, combined with heat-induced cross-linking, which remain insoluble (Kemppainen *et al.*, 2016; Rommi *et al.*, 2018). BSG proteins can also bind with lignin in SE HTT as pseudo lignin that increase the insoluble lignin fraction (Rommi *et al.*, 2018).

Higher temperature treatment in SE (B-4) at 200 °C further increased essential amino acid content to 43.4% (Table 5-5). Results from the SE residues for WBSG-25% (run A-4) and for PBSG-25% (run B-2) show the essential amino acids increased to 40.7% and 39.5% respectively, which selectively reduced basic amino acids while increasing hydrophobic amino acids. SO_2 catalyst addition to SE (run B-7) resulted in decreased methionine and no significant change in essential amino acid concentration was observed compared to run B-2 (no SO_2). Overall, the amino acid profile of the PBSG was found to be similar to reported values (Connolly, 2013) consisting mostly of hydrophobic amino acids (50%). However, the most abundant amino acid was found to be hydrophilic glutamine of 20.5% (Table 5-5). WBSG was found to show similar amino acid profile trends but with slight variation from the pure barley PBSG with 23.2% glutamine. While WBSG compared to PBSG had similar amounts of essential amino acids (37.3% and 36.8%), the hydrophilic amino acids in WBSG was slightly higher (44.2%) compared to PBSG (40.3%).

Table 5-5 Amino acid profile of WBSG and PBSG, press liquid IS, SE residues and SE hydrolysates

Amino acid[a] (wt %)	Feedstock		Screw press liquid product IS		Steam explosion solid residues				Steam explosion hydrolysate PBSG			
					WBSG-25	PBSG-25%			Free amino acids (Total as µg/g dm)		Total amino acids	
	WBSG-15%	PBSG-15%	WBSG	PBSG	180°C 10min A-4	180°C 10min B-2	200°C 5min B-4	180°C w SO_2 10min B-7	180°C 10min B-2	200°C 5min B-4	180°C 10min B-2	200°C 5min B-4
Basic amino acids												
Arginine	6.5%	5.6%	5.6%	4.7%	2.6%	0.0%	1.3%	2.2%	0.9%	2.8%	2.7%	1.3%
Histidine*	3.2%	2.7%	1.5%	2.4%	2.9%	2.2%	2.7%	2.0%	n.d.	3.4%	3.1%	3.1%
Lysine*	2.3%	5.5%	3.5%	3.7%	0.9%	1.5%	0.5%	2.3%	0.0%	0.0%	0.7%	0.0%
Subtotal	**12.0%**	**13.8%**	**10.5%**	**10.8%**	**6.4%**	**3.7%**	**4.5%**	**5.5%**	**0.9%**	**6.2%**	**6.5%**	**4.4%**
Hydrophobic amino acids												
Alanine	4.5%	5.0%	4.8%	4.8%	5.7%	7.7%	6.4%	7.4%	4.3%	5.7%	5.8%	5.8%
Glycine	4.9%	4.7%	4.4%	3.9%	5.2%	5.7%	5.9%	5.7%	4.4%	6.5%	6.3%	6.2%
Isoleucine*	3.7%	3.8%	4.1%	3.8%	4.3%	4.8%	4.6%	5.0%	1.0%	1.8%	3.6%	3.3%
Leucine*	7.8%	7.8%	7.8%	7.4%	9.6%	9.8%	10.5%	10.3%	2.0%	3.3%	6.2%	6.3%
Methionine*	2.4%	2.7%	2.2%	1.8%	3.0%	3.9%	3.3%	1.3%	n.d.	n.d.	2.3%	2.2%
Phenylalanine*	7.5%	5.2%	5.1%	5.6%	8.6%	6.1%	9.6%	6.6%	n.d.	n.d.	6.3%	6.9%
Proline	7.2%	11.7%	12.2%	13.6%	6.9%	11.1%	7.5%	12.5%	2.5%	5.1%	8.5%	9.5%
Valine*	5.8%	5.1%	5.4%	4.7%	6.5%	6.2%	7.0%	6.6%	7.1%	9.2%	5.6%	5.7%
Subtotal	**43.8%**	**45.9%**	**46.0%**	**45.6%**	**49.7%**	**55.4%**	**54.8%**	**55.4%**	**21.3%**	**31.6%**	**44.7%**	**45.7%**
Hydrophilic amino acids												
Asparagine	6.3%	8.3%	7.3%	8.0%	5.2%	6.8%	4.1%	4.7%	71.7%	51.4%	8.7%	4.9%
Glutamine	23.2%	20.5%	24.4%	24.2%	22.7%	20.7%	20.1%	20.6%	0.0%	0.0%	26.0%	30.8%
Serine	5.8%	4.3%	4.6%	4.3%	5.9%	5.0%	5.7%	5.0%	2.1%	1.7%	5.2%	5.4%
Threonine*	4.6%	4.1%	3.8%	3.8%	4.9%	4.9%	5.3%	4.7%	0.3%	0.0%	4.5%	4.2%
Tyrosine	4.1%	3.2%	3.4%	3.4%	5.1%	3.6%	5.6%	4.1%	3.8%	9.1%	4.3%	4.5%
Subtotal	**44.2%**	**40.3%**	**43.4%**	**43.7%**	**43.9%**	**40.9%**	**40.8%**	**39.1%**	**77.9%**	**62.2%**	**48.8%**	**49.8%**
Essential amino acids*												
Subtotal included	**37.3%**	**36.8%**	**33.4%**	**33.1%**	**40.7%**	**39.5%**	**43.4%**	**37.8%**	**10%**	**18%**	**21%**	**20%**
Total amino acids	**23.3%**	**25.6%**	**43.0%**	**50.6%**	**22.6%**	**17.3%**	**14.4%**	**17.3%**	349.84	141.17	**10.3%**	**8.6%**
Crude Protein (N x 6.25)	24.3%	21.5%	39.5%	46.5%	25.1%	19.8%	19.6%	19.2%			15.3%	-

a Tryptophan and Cysteine not determined;

5.4 Conclusions

XOS production from BSG for application in novel food and beverages can reduce brewery waste streams and increase resource efficiency. XOS production optimisations reported in bench scale LHW HTT of BSG can be used for scale-up to pilot scale SE, achieving similar and higher XOS yields (>73%) by using >60% less water. Moisture content was shown as an important HTT optimisation variable. Small variations in BSG compositions did not significantly affect near optimal conditions. SE severity adjustment through SO_2, catalyst addition or process conditions can increase the valuable short chain X2 and X3 oligomers in the XOS up to 31.3%.

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E-supplementary data of this work can be found in online version of the paper.

5.6 References

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: Fundamentals toward application. Biotechnol. Adv. 29, 675–685. https://doi.org/10.1016/j.biotechadv.2011.05.005

Allen, Stephen Glen, Schulman, D., Lichwa, J., Antal, M.J., Jennings, E., Elander, R., 2001. A Comparison of Aqueous and Dilute-Acid Single-Temperature Pretreatment of Yellow Poplar Sawdust. Ind. Eng. Chem. Res. 2352–2361.

Allen, S G, Schulman, D., Lichwa, J., Antal, M.J., Laser, M., Lynd, L.R., 2001. A comparison between hot liquid water and steam fractionation of corn fiber. Ind. Eng. Chem. Res. 40, 2934–2941. https://doi.org/10.1021/ie990831h

Amorim, C., Silvério, S.C., Rodrigues, L.R., 2019. One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. Food Chem. 270, 86–94. https://doi.org/10.1016/j.foodchem.2018.07.080

Barata, A., Caldeira, J., Botelheiro, R., Pagliara, D., Malfeito-Ferreira, M., Loureiro, V., 2008. Survival patterns of Dekkera bruxellensis in wines and inhibitory effect of sulphur dioxide. Int. J. Food Microbiol. 121, 201–207. https://doi.org/10.1016/j.ijfoodmicro.2007.11.020

Blinkov, S.D., Nemoytin, J.B.B.M., Fedorov, A.L., 1999. Method for processing vegetal raw materials in order to produce pentose hydrolysates essentially containing xylose. WO1999023260.

Brownell, H.H., Saddler, J.N., 1987. Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. Biotechnol. Bioeng. 29, 228–235. https://doi.org/10.1002/bit.260290213

Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam explosion pretreatment of wood: Effect of Chip Size, Acid, Moisture Content and Pressure Drop. Biotechnol. Bioeng. 28, 792–801. https://doi.org/10.1002/bit.260280604

Carvalheiro, F., Esteves, M.P., Parajo, J.C., Pereira, H., Gıırio, F.M., 2004. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91, 93–100. https://doi.org/10.1016/S0960-8524(03)00148-2

Carvalho, A.F.A., Marcondes, W.F., de Oliva Neto, P., Pastore, G.M., Saddler, J.N., Arantes, V., 2018. The potential of tailoring the conditions of steam explosion to produce xylo-oligosaccharides from sugarcane bagasse. Bioresour. Technol. 250, 221–229. https://doi.org/10.1016/j.biortech.2017.11.041

Celus, I., Brijs, K., Delcour, J.A., 2009. Fractionation and characterization of brewers' spent grain protein hydrolysates. J. Agric. Food Chem. 57, 5563–5570. https://doi.org/10.1021/jf900626j

Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst effects and the combined severity parameter. Appl. Biochem. Biotechnol. 24–25, 1–14. https://doi.org/10.1007/BF02920229

Church, J.A., Wooldridge, D., 1981. Continuous High-Solids Acid Hydrolysis of Biomass in a 11/2-in. Plug Flow Reactor. Ind. Eng. Chem. Prod. Res. Dev. 20, 371–378. https://doi.org/10.1021/i300002a027

Cian, R.E., Garzón, A.G., Martínez-augustin, O., Botto, C.C., Drago, S.R., 2018. Antithrombotic Activity of Brewers' Spent Grain Peptides and their Effects on Blood Coagulation Pathways. J. Supercrit. Fluids 140, 85–90.

Connolly, A., 2013. Generation and characterisation of novel health enhancing ingredients from brewers ' spent grain. University of Limerick.

Connolly, A., O'Keeffe, M.B., Nongonierma, A.B., Piggott, C.O., FitzGerald, R.J., 2017. Isolation of peptides from a novel brewers spent grain protein isolate with potential to modulate glycaemic response. Int. J. Food Sci. Technol. 52, 146–153. https://doi.org/10.1111/ijfs.13260

Corte, L., Roscini, L., Zadra, C., Antonielli, L., Tancini, B., Magini, A., Emiliani, C., Cardinali, G., 2012. Effect of pH on potassium metabisulphite biocidic activity against yeast and human cell cultures. Food Chem. 134, 1327–1336. https://doi.org/10.1016/j.foodchem.2012.03.025

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of Initial Moisture Content and Chip Size on the Bioconversion Efficiency of Softwood Lignocellulosics. Biotechnol. Bioeng. 85, 413–421. https://doi.org/10.1002/bit.10905

García-Aparicio, M., Parawira, W., Van Rensburg, E., Diedericks, D., Galbe, M., Rosslander, C., Zacchi, G., Görgens, J., 2011. Evaluation of steam-treated giant bamboo for production of fermentable sugars. Biotechnol. Prog. 27, 641–649. https://doi.org/10.1002/btpr.580

Garrote, G., Domı, H., Parajo, J.C., 2004. Production of Substituted Oligosaccharides by Hydrolytic Processing of Barley Husks. Ind. Eng. Chem. Res. 43, 1608–1614.

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

Gullón, P., González-Muñoz, M.J., Parajó, J.C., 2011. Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. Bioresour. Technol. 102, 6112–6119. https://doi.org/10.1016/j.biortech.2011.02.059

Gurgel, L.V.A., Pimenta, M.T.B., Curvelo, A.A. da S., 2014. Enhancing liquid hot water (LHW) pretreatment of sugarcane bagasse by high pressure carbon dioxide (HP-CO2). Ind. Crops Prod. 57, 141–149. https://doi.org/10.1016/j.indcrop.2014.03.034

Heitz, M., Capek-Ménard, E., Koeberle, P.G., Gagné, J., Chornet, E., Overend, R.P., Taylor, J.D., Yu, E., 1991. Fractionation of Populus tremuloides at the pilot plant scale: Optimization of steam pretreatment conditions using the STAKE II technology. Bioresour. Technol. 35, 23–32. https://doi.org/10.1016/0960-8524(91)90078-X

Huige, N.J., 1994. Handbook of Brewing: In Food Science and Technology, in: Hardwick, W.A. (Ed.), Marcel Dekker Inc. CRC Press, pp. 501–550). https://doi.org/10.1002/9780470290118

Ibbett, R., Gaddipati, S., Davies, S., Hill, S., Tucker, G., 2011. The mechanisms of hydrothermal deconstruction of lignocellulose : New insights from thermal – analytical and complementary studies. Bioresour. Technol. 102, 9272–9278. https://doi.org/10.1016/j.biortech.2011.06.044

Ikram, S., Huang, L., Zhang, H., Wang, J., Yin, M., 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 00, 1–11. https://doi.org/10.1111/1750-3841.13794

Ishiwaki, B.N., Murayama, H., Awayama, H., Kanauchi, O., Sato, T., 2000. Development of High Value Uses of Spent Grain by Fractionation Technology Q uarterly. MBAA Tech. Q. 37, 261–265.

Jacobsen, S.E., Wyman, C.E., 2002. Xylose Monomer and Oligomer Yields for Uncatalyzed Hydrolysis of Sugarcane Bagasse Hemicellulose at Varying Solids Concentration. Ind. Eng. Chem. Res. 41, 1454–1461. https://doi.org/10.1021/ie001025+

Jacobsen, S.E., Wyman, C.E., 2000. Cellulose and Hemicellulose Hydrolysis Models for Application to Current and Novel Pretreatment Processes. Appl. Biochem. Biotechnol. 84–86, 81–96. https://doi.org/10.1385/ABAB:84-86:1-9:81

Jay, A.J., Parker, M.L., Faulks, R., Husband, F., Wilde, P., Smith, A.C., Faulds, C.B., Waldron, K.W., 2008. A systematic micro-dissection of brewers' spent grain. J. Cereal Sci. 47, 357–364. https://doi.org/10.1016/j.jcs.2007.05.006

Kemppainen, K., Rommi, K., Holopainen, U., Kruus, K., 2016. Steam explosion of Brewer's spent grain improves enzymatic digestibility of carbohydrates and affects solubility and stability of proteins. Appl. Biochem. Biotechnol. 1–15. https://doi.org/10.1007/s12010-016-2085-9

Kerby, C., Vriesekoop, F., 2017. An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3, 1–12. https://doi.org/10.3390/beverages3020024

Laser, M., Schulman, D., Allen, S.G., Lichwa, J., Antal, M.J., Lynd, L.R., 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour. Technol. 81, 33–44. https://doi.org/10.1016/S0960-8524(01)00103-1

Liao, W., Liu, Y., Liu, C., Chen, S., 2004. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material - Dairy manure. Bioresour. Technol. 94, 33–41. https://doi.org/10.1016/j.biortech.2003.11.011

Lischeske, J.J., Crawford, N.C., Kuhn, E., Nagle, N.J., Schell, D.J., Tucker, M.P., McMillan, J.D., Wolfrum, E.J., 2016. Assessing pretreatment reactor scaling through empirical analysis. Biotechnol. Biofuels 9, 213. https://doi.org/10.1186/s13068-016-0620-0

López-Linares, J.C., García-Cubero, M.T., Lucas, S., González-Benito, G., Coca, M., 2019. Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production. Chem. Eng. J. 368, 1045–1055. https://doi.org/10.1016/j.cej.2019.03.032

Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. J. Inst. Brew. 4, 553–568. https://doi.org/10.1002/jib.363

Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment-A review. Biotechnol. Bioeng. 109, 1430–1442. https://doi.org/10.1002/bit.24464

Morinelly, J.E., Jensen, J.R., Browne, M., Co, T.B., Shonnard, D.R., 2009. Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass. Ind. Eng. Chem. Res. 48, 9877–9884. https://doi.org/10.1021/ie900793p

Mosier, N.S., Ladisch, C.M., Ladisch, M.R., 2002. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. Biotechnol. Bioeng. 79, 610–618. https://doi.org/10.1002/bit.10316

Mussatto, S.I., 2014. Brewer's spent grain: A valuable feedstock for industrial applications. J. Sci. Food Agric. 94, 1264–1275. https://doi.org/10.1002/jsfa.6486

Mussatto, S.I., Roberto, I.C., 2006. Chemical characterization and liberation of pentose sugars from brewer's spent grain. J. Chem. Technol. Biotechnol. 81, 268–274. https://doi.org/10.1002/jctb.1374

Negahdar, L., Delidovich, I., Palkovits, R., 2016. Aqueous-phase hydrolysis of cellulose and hemicelluloses over molecular acidic catalysts: Insights into the kinetics and reaction mechanism. Appl. Catal. B Environ. 184, 285–298.

Nigam, P.S., 2017. An overview: Recycling of solid barley waste generated as a by-product in distillery and brewery. Waste Manag. 62, 255–261. https://doi.org/10.1016/j.wasman.2017.02.018

Öhgren, K., Galbe, M., Zacchi, G., 2005. Optimization of Steam Pretreatment of SO 2 - Impregnated Corn Stover for Fuel Ethanol Production. Appl. Biochem. Biotechnol. 121.

Olsson, L., 2007. Biofuels, Advances in Biochemical engineering/Biotechnology. https://doi.org/10.1016/j.jbiotec.2007.09.006

Overend, R., Chornet, E., Gascoigne, J.A., 1987. Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments [and Discussion], Philosophical Transactions of The Royal Society A: Mathematical, Physical and Engineering Sciences. https://doi.org/10.1098/rsta.1987.0029

Pedersen, M., Johansen, K.S., Meyer, A.S., 2011. Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw. Biotechnol. Biofuels 4, 11. https://doi.org/10.1186/1754-6834-4-11

Pedersen, M., Meyer, A.S., 2010. Lignocellulose pretreatment severity - relating pH to biomatrix opening. N. Biotechnol. 27, 739–750. https://doi.org/10.1016/j.nbt.2010.05.003

Pires, E.J., Ruiz, H.A., Teixeira, J.A., Vicente, A.A., 2012. A new approach on brewer's spent grains treatment and potential use as lignocellulosic yeast cells carriers. J. Agric. Food Chem. 60, 5994–5999. https://doi.org/10.1021/jf300299m

Qihua, D., Li, F., Deliang, W., Xia, L., Zhiping, L., Fengchao, J., 2010. Research into Pretreatment Methods of Brewer's Spent Grain (Chinese). Food Ferment. Ind. 36, 116–121.

Robertson, J.A., I'Anson, K.J.A., Treimo, J., Faulds, C.B., Brocklehurst, T.F., Eijsink, V.G.H., Waldron, K.W., 2010. Profiling brewers' spent grain for composition and microbial ecology at the site of production. LWT - Food Sci. Technol. 43, 890–896. https://doi.org/10.1016/j.lwt.2010.01.019

Rojas Pérez, L.C., 2018. Valorisation of brewers spent grains in the production of xylitol (Spanish). Universidad Nacional de Colombia.

Rommi, K., Niemi, P., Kemppainen, K., Kruus, K., 2018. Impact of thermochemical pre-treatment and carbohydrate and protein hydrolyzing enzyme treatment on fractionation of protein and lignin from brewer's spent grain. J. Cereal Sci. 79, 168–173. https://doi.org/10.1016/j.jcs.2017.10.005

Roos, A.A., Persson, T., Krawczyk, H., Zacchi, G., Stålbrand, H., 2009. Extraction of water-soluble hemicelluloses from barley husks. Bioresour. Technol. 100, 763–769. https://doi.org/10.1016/j.biortech.2008.07.022

Shen, J., Wyman, C.E., 2011. A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. Bioresour. Technol. 102, 9111–9120. https://doi.org/10.1016/j.biortech.2011.04.001

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J. Agric. Food Chem. 58, 9043–9053. https://doi.org/10.1021/jf1008023

Stiles, R.S., Herbert, H.S., 1977. Centrifugal Separation, Isolation and Characteristics of Brewery Spent Grains and Press Liquor. MBAA Tech. Q. 14, 21–34.

Sui, W., Chen, H., 2015. Study on loading coefficient in steam explosion process of corn stalk. Bioresour. Technol. 179, 534–542. https://doi.org/10.1016/j.biortech.2014.12.045

Tucker, M.P., Nagle, N.J., Jennings, E.W., Ibsen, K.N., Aden, A., Nguyen, Q.A., Kim, K.H., Noll, S.L., 2004. Conversion of distiller's grain into fuel alcohol and a higher-value animal feed

by dilute-acid pretreatment. Appl Biochem Biotechnol 113–116, 1139–1159. https://doi.org/10.1385/ABAB:115:1-3:1139

Vallejos, M.E., Zambon, M.D., Area, M.C., Curvelo, A.A. da S., 2012. Low liquid–solid ratio (LSR) hot water pretreatment of sugarcane bagasse. Green Chem. 14, 1982. https://doi.org/10.1039/c2gc35397k

Wang, L., Fan, X., Tang, P., Yuan, Q., 2013. Xylitol fermentation using hemicellulose hydrolysate prepared by acid pre-impregnated steam explosion of corncob. J. Chem. Technol. Biotechnol. 88, 2067–2074. https://doi.org/10.1002/jctb.4070

Weber, B., Stadlbauer, E.A., 2017. Sustainable paths for managing solid and liquid waste from distilleries and breweries. J. Clean. Prod. 149, 38–48. https://doi.org/10.1016/j.jclepro.2017.02.054

Weger, A., Binder, S., Franke, M., Hornung, A., 2014. Solid Biofuel Production by Mechanical Pre-Treatment of Brewers' Spent Grain. Chem. Eng. Trans. 37, 661–666. https://doi.org/10.3303/CET1437111

WHO, 2012. S.3.6. Bulk Density and Tapped Density of Powders QAS/11.450 FINAL. https://doi.org/10.1007/s13398-014-0173-7.2

Yan, Q., Miazek, K., Grande, P.M., Domínguez de María, P., Leitner, W., Modigell, M., 2014. Mechanical pretreatment in a screw press affecting chemical pulping of lignocellulosic biomass. Energy & Fuels 28, 6981–6987. https://doi.org/10.1021/ef501706w

Yang, B., Gray, M.C., Liu, C., Lloyd, T.A., Stuhler, S.L., Converse, A.O., Wyman, C., 2004. Unconventional Relationships for Hemicellulose Hydrolysis and Subsequent Cellulose Digestion, in: Lignocellulose Biodegradation. American Chemical Society..

Appendix: Chapter 5 - Steam explosion HTT experimental data

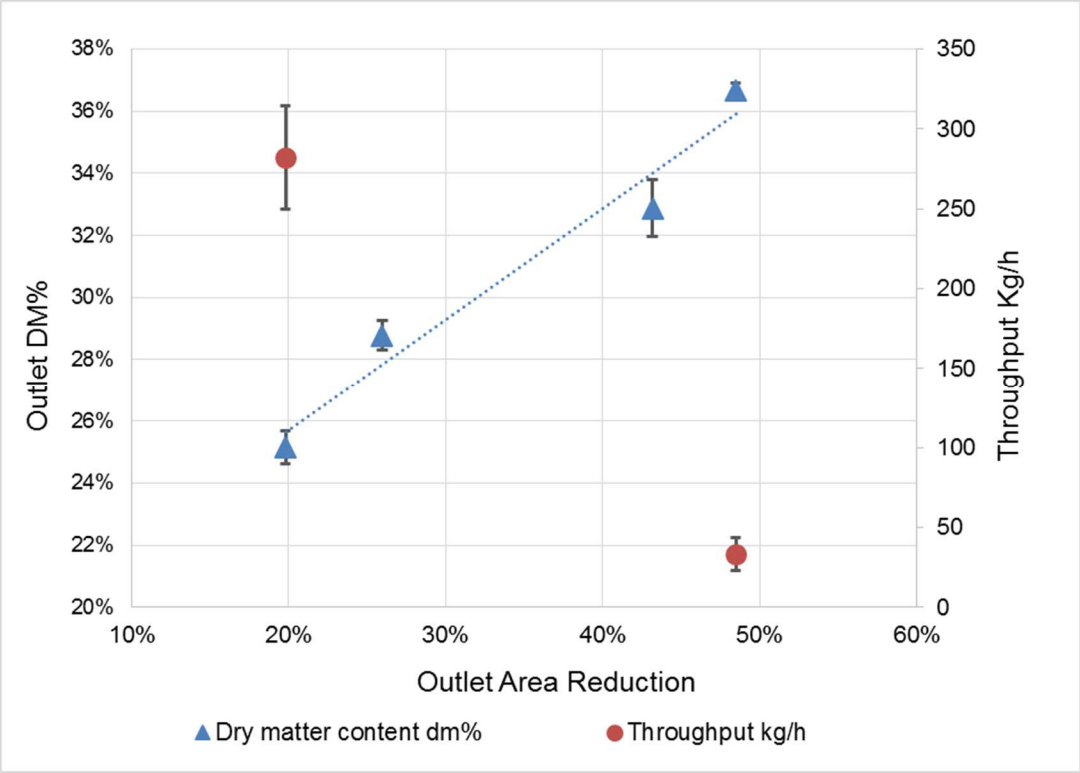

Figure 5A-1 Screw press operation for PBSG: Throughput and dry matter content yield

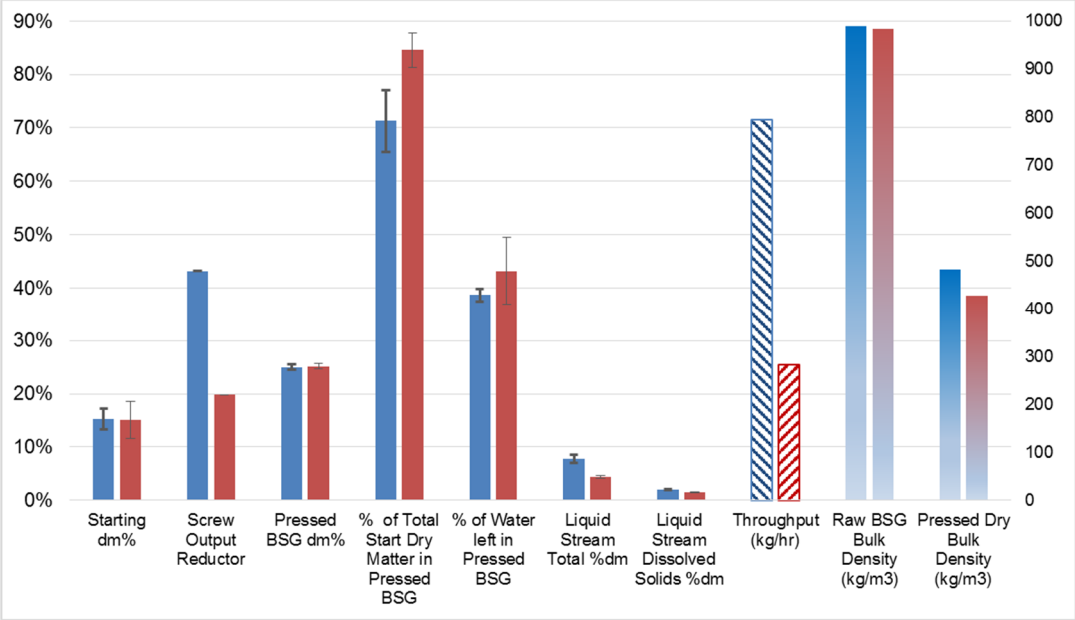

Figure 5A-2 Screw press operation comparison for WBSG and PBSG 15% to 15% dry matter: Mass balance and throughput

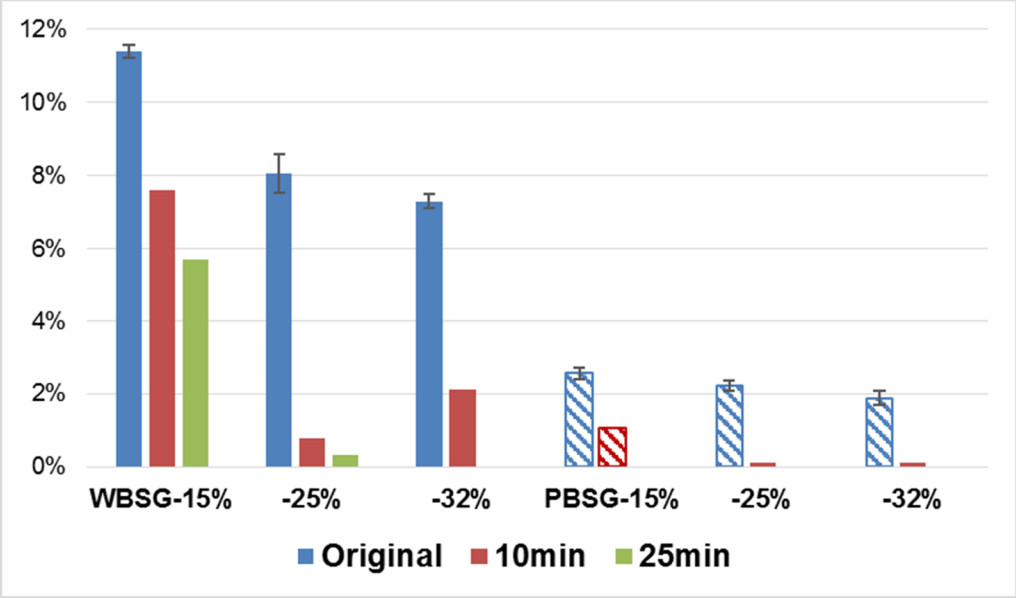

Figure 5A-3 Residual insoluble starch after steam explosion of WBSG and PBSG

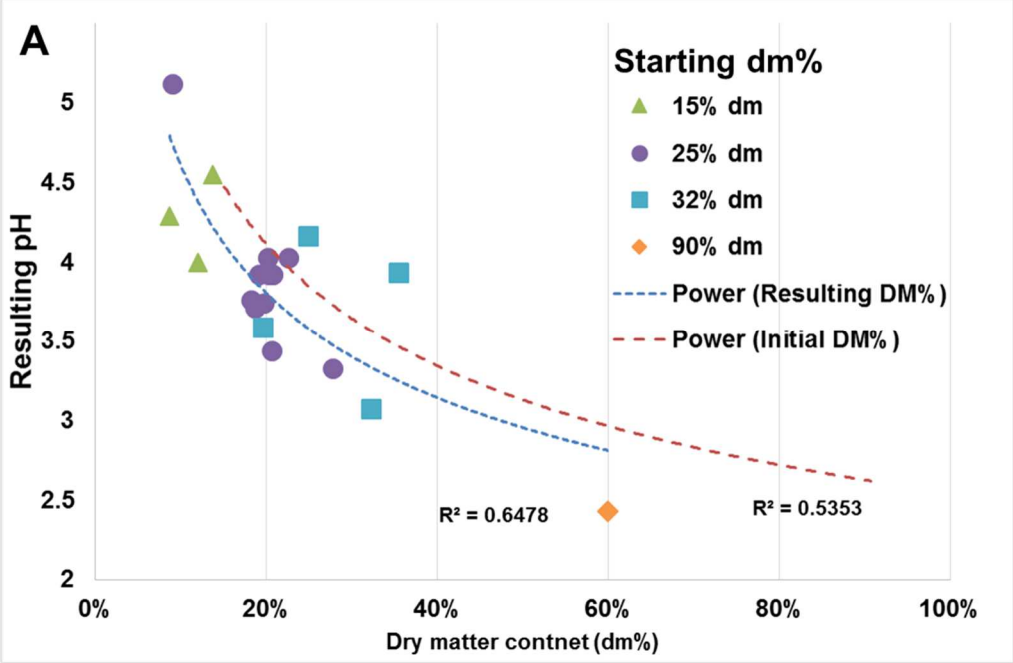

Figure 5A-4 Initial dry matter content and the dilution effect on resulting pH in steam explosion

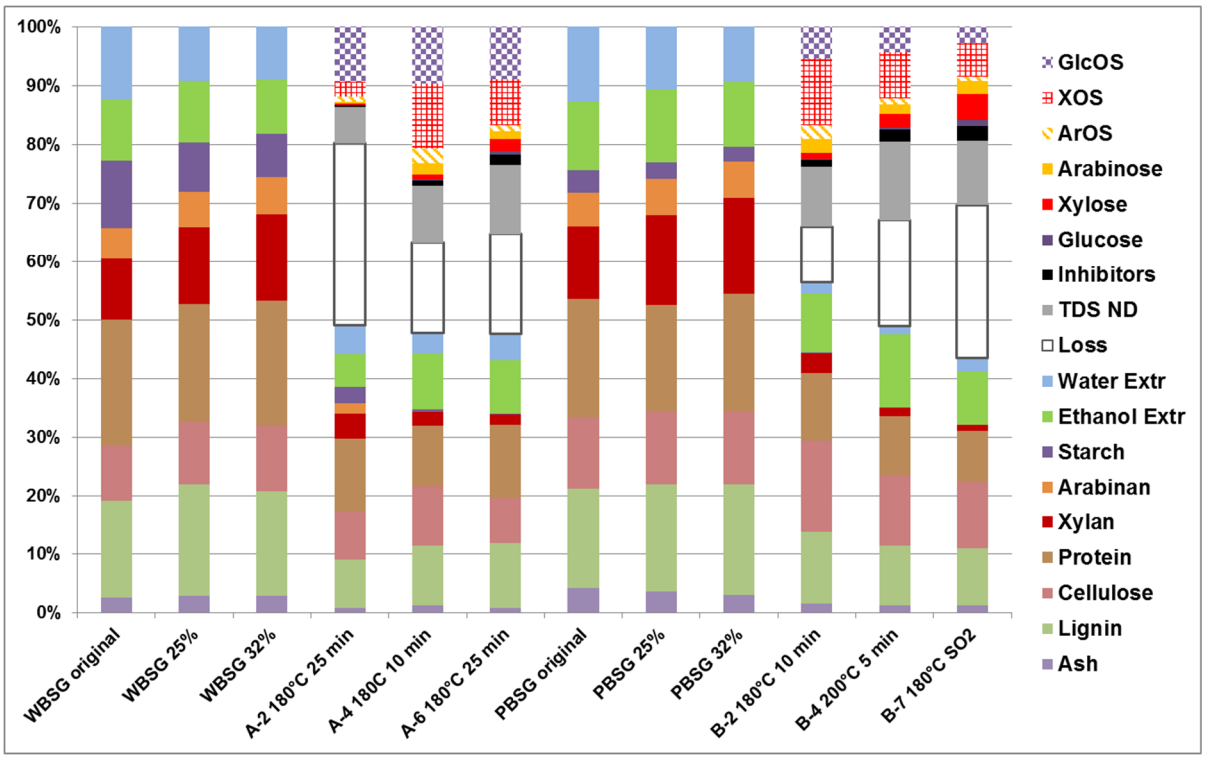

Figure 5A-5 Steam explosion insoluble residue from WBSG and PBSG

Table 5A-1 Comparison on proteins removal/splits in SE process step

Process step	Protein in solid remaining (%)		Difference (liquid or lost)
1.Screw Press			
PBSG-15% to PBSG-25%	71.6		28.4
2.SE of PBSG-25%		**(of 71.6)**	
SE 180 °C 10 min	45.3	63.2%	26.4
SE 200 °C 5 min	39.4	54.9%	32.3
SE 180 °C 10 min SO_2	34.3	47.9%	37.3

Table 5A-2 Acid insoluble (AI) solid fraction crude nitrogen content in SE solid residues

BSG feedstock	Steam explosion		Acid Insoluble (AI) %		
	Temp (°C)	Time (min)	AI Fraction	AI Protein	AI Ash
PBSG-15%			**24.5**	**46.2**	**3.8**
PBSG-32%			**26.5**	**40.4**	**5.3**
PBSG-25%	180	10	33.2	39.6	6.0
PBSG-25%	200	5	32.4	39.4	5.4
PBSG-25% SO_2	180	10	33.4	35.7	5.5
WBSG-15%			**22.4**	**38.2**	**1.8**
WBSG-15%	180	25	26.9	45.0	2.7
WBSG-25%	180	10	33.7	47.1	2.2
WBSG 25%	180	25	34.5	37.7	1.3

6. Techno-economic analysis of the valorisation of brewers spent grains: Production of xylitol and xylo-oligosaccharides

Contribution to overall scope of work

In this paper (Chapter 6) the concept of valorisation of BSG by production of XOS and xylitol through HTT was investigated. This study included the HTT process intensification achieved with HTT experiments in Chapter 4 and 5 that demonstrated a >60% reduction in water and reduced process temperatures with similar or higher XOS yields than reported previously in literature. In Chapter 4 processing of BSG in the stirred batch LHW HTT with ELA dosing of between 5 and 20 mg H_2SO_4/g dm, obtained XOS yields between 65.5%-76.4% higher or similar than previous reported autocatalytic LHW HTT processes (61-77%). Yet the tested conditions with 46 mg H_2SO_4/g dm at 120 ºC achieved >85% total xylan recovery with *circa* 50/50 split as xylose and XOS and the XOS contained the highest fraction (>30%) short chain xylobiose (X2) and xylotriose (X3) from the tested conditions, which is a XOS best suitable for a prebiotic XOS product. In Chapter 5, scaled-up in pilot steam explosion HTT a preferred process condition (180 ºC and 10 min) achieved XOS yields between 73.1%-75.3% (79.3%-81.1% xylan recovery). These improvements in HTT technology of BSG were applied in techno-economic assessment of three concept processes for valorisation of BSG to produce XOS, xylitol and both together, at a scale applicable to a large brewery. Results found IRR greater than the hurdle rate (9.7%) for all scenarios when considering a conservative market price for xylitol and XOS as US$4500/t, yet production of XOS was economically more favourable with a MRSP of US$2509/t compared to US$4153/t for xylitol. Additionally the production of both products xylitol and XOS together achieved the lowest MRSP of US$2182/t.

In conclusion, the second overall research objective described for the techno-economic evaluation of novel processes in ASPEN simulations for the production of xylitol and XOS from BSG when extracting protein by-product with screw press dewatering process step using the experimental results from improved HTT of BSG conducted in Chapter 4 and 5. This objective was completed in this Chapter 6.

Techno-economic analysis of the valorisation of brewers spent grains: Production of xylitol and xylo-oligosaccharides

This Chapter is prepared in article format according to the journal requirements for publication in *Biofuels, Bioproducts and Biorefining* from John Wiley & Sons Ltd (London, United Kingdom).

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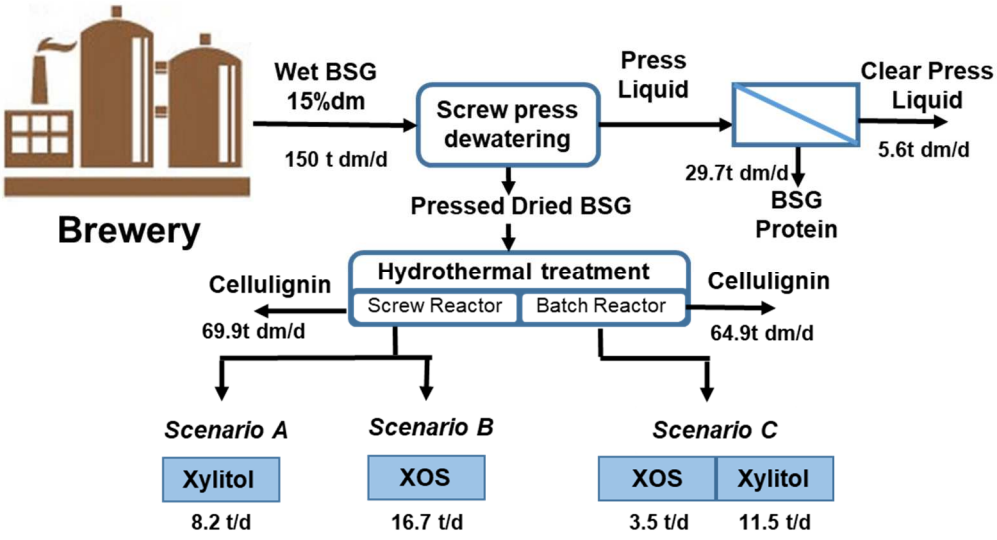
Authors' contribution

LJ is the primary author and investigator of the paper, Abdul gave technical guide during the simulations development and write-up, was the internal reviewer and assisted in the structuring of the paper. JG is the study leader and contributed to the final review and approved the paper for submission for publication. All authors provided input to the manuscript and read and approved the final manuscript.

Abstract

Brewers spent grains (BSG) represents up to 85% of a brewery's solid waste and common disposal to landfill or routing farmers is increasingly more difficult. Yet BSG is a food grade by-product with potential economic valorisation that can provide significant improved resource efficiency and reduction in carbon emissions. The study investigated valorisation of BSG in three scenarios: (i) the production of sugar replacer xylitol, (ii) prebiotic xylo-oligosaccharide (XOS) and (iii) both together through the application of novel high solids hydrothermal processing technology in a small scale biorefinery approach, annexed to a brewery. Economic assessment was conducted by comparing the capital and operating from process simulations created in ASPEN Plus®. Process models developed were supplemented with experimental data to improve accuracy. Results found IRR values greater than the hurdle rate of 9.7% for all scenarios when considering a conservative market price for xylitol and XOS as US$4500/t, yet dedicated production of XOS was economically more favourable with a MRSP of US$2509/t compared to US$4153/t for xylitol. Additionally, the scenario for co-production of xylitol and XOS together achieved the lowest MRSP of US$2182/t. By-products significantly contributed to the revenue that comprised 32.7%, 14.2% and 27.5% for xylitol, XOS and the combination respectively.

Keywords: Brewer's spent grains; small scale biorefinery; techno- economic assessment; food waste; circular economy; profitability; xylitol; xylo-oligosaccharides



Highlights

- All scenarios for BSG valorisation demonstrated profitability even at a small production scale
- Dedicated production of XOS was more economically favourable compared to xylitol
- A multiproduct scenario delivered increased IRR

6.1 Introduction

Beer is an alcoholic beverage produced from grains like malted barley, wheat or rice. It is the third most popular drink after water and tea (Oliver, 2011) with a global consumption rate of 45.7 litre per adult per year (The Economist, 2017). The solid residue, brewers' spent grains (BSG) is left after saccharification of these starch from these grains, and is generated at about 19 kg wet residue per 100 L beer (Mussatto, 2014). The BSG contains between 70% to 85% water and is typically disposed of without drying (Lynch, Steffen and Arendt, 2016). A typical medium size brewery can produce 100 to 200 tonnes wet BSG per day and the largest brewery in South Africa produces nearly 500 tonnes of wet BSG per day, or on a dry basis, up to 150 tonnes per day (Ishiwaki *et al.*, 2000; Ramukhwatho, Seetal and Pienaar, 2016). Due to its warm temperature (>40 ºC) when discharged, high moisture content and the contents of bioactive organic matter (Robertson, I'Anson, *et al.*, 2010), the material is highly susceptible to spoiling and cannot be stored for long periods (Wang *et al.*, 2014). Therefore, it is a potentially bio-hazardous waste and its timely disposal can be a logistical problem to large breweries (Huige, 1994; Ramukhwatho, Seetal and Pienaar, 2016). Currently, most BSG is either sent to landfill or transported to nearby farmers as an animal feed supplement (Schwencke, 2006; Kerby and Vriesekoop, 2017). Yet with declining numbers of feedlots in developing countries (Ishiwaki *et al.*, 2000; Weger *et al.*, 2014), increasingly stringent regulations on animal feeds (Kerby and Vriesekoop, 2017), coupled with the drive to create more sustainable processes with reduced CO_2 emissions (Monin, 2016) have compelled breweries to find alternative solutions for BSG valorisation and disposal. Appropriately, research efforts have investigated alternative solutions in biochemicals and food products derived from BSG with re-application in brewing, food, energy, cosmetic and pharmaceutical industries (Mussatto, 2014; Skendi, Harasym and Galanakis, 2018).

The high moisture content of BSG results in high transportation costs of BSG, which hinders economically viable methods of BSG valorisation, especially for commodity products like biofuels that require large scale biorefinery concepts with more than 2000 tonnes dry per day (Buffington, 2014), and thus, higher value products are needed. Accordingly, recent studies on BSG valorisation have focused on health-promoting food product applications including antioxidants, protein/peptide concentrates, prebiotics, low glycaemic arabinose sugars and sugar replacers xylitol as alternative applications for higher value valorisation propositions (Lynch, Steffen and Arendt, 2016; Connolly *et al.*, 2017; Ikram *et al.*, 2017). Extraction of such high value compounds from BSG may provide support for a valorisation case even in a small scale (<2000 tonne dry biomass per day) biorefinery by rendering it economically feasible. High value products with large profit margins, such as xylitol (Mussatto *et al.*, 2013), can

mitigate the negative cost impact from reduced economy of scale with a typical brewery BSG production capacity (Bruins and Sanders, 2012).

The earliest examples of BSG valorisation for food formulation have focused on the extraction of the BSG protein. BSG is a sustainable alternative plant protein source since it contains up to 25% protein (Schwencke, 2006; Weger *et al.*, 2017), from which recently bioactive peptides were obtained (Connolly *et al.*, 2017; Cian *et al.*, 2018). Sustainable plant base proteins sources are increasingly valued as a result of a growing interest for replacement of animal based proteins (Ercili *et al.*, 2018). Mechanical methods such as a screw press can produce a BSG fraction high in protein (>50%) with a remaining fraction enriched in fibre ('Patent WO1999023260A1 - Method for proc...o produce pentose Blinkov 1999.pdf', no date; Ishiwaki *et al.*, 2000) that can be either used for low value fuel (Schwencke, 2006; Weger *et al.*, 2017) or high fibre filler (Züricher and Gruss, 1990; Ishiwaki *et al.*, 2000). Yet this fibre fraction is ideal for extraction of high value compounds.

BSG fibre has a large hemicellulose fraction (>25%), mainly made up of xylan and arabinan making it a suitable source for production of xylo-oligosaccharides (XOS) and xylose sugars leading to xylitol (Carvalheiro et al., 2004b; Gómez et al., 2015; Mussatto and Roberto, 2005). Xylitol is a naturally occurring sugar alcohol and has a third fewer calories for the equivalent sweetness of sucrose with low glycaemic response suitable for diabetics (Ur-Rehman *et al.*, 2013). On the other hand, XOS are dietary fibres composed of mainly water soluble xylan oligomers that have a degree of polymerisation (DP) <30 and are valued for their reported beneficial health effects, in particular, the prebiotic effects (Vazquez *et al.*, 2000; Carvalho *et al.*, 2013). Moreover, arabinose substituted XOS are more desirable and BSG is more suitable for their production with its higher arabinan content compared to other sources (Gomez *et al.*, 2015).

The extraction and (partial) depolymerisation of xylan for either/both XOS and xylitol production can be achieved by hydrothermal treatment (HTT) at appropriate process conditions (Carvalheiro *et al.*, 2004). Xylitol production from xylose is currently largely by high temperature chemical synthesis (Özüdoğru *et al.*, 2019), however, the more desired and eco-friendly biotechnological route is mostly pursued by xylose fermentation, where the fermented xylitol broth is purified by chromatographic separation to remove inhibitors and concentrated before crystallisation of pure xylitol crystals (Da Silva and Chandel, 2012). On the other hand, XOS production involves fewer processing steps. The extracted xylan XOS oligomers in the hydrolysate is partially depolymerised to short chain XOS of degree of polymerisation (DP) <10, purified by membrane filtration and adsorption before drying to produce XOS (Carvalheiro *et al.*, 2004; Gullón, González-Muñoz and Parajó, 2011; Gomez *et al.*, 2015). Nevertheless, both xylitol and XOS are high value speciality chemicals with values >US$ 5/kg

with projected markets with compound annual growth rate (CAGR) >6% (Llzo and Lx, no date; Biddy *et al.*, 2016).

A techno-economic evaluation of BSG valorisation into xylitol, lactic acid, phenolic acids and activated carbon in Brazil, concluded that xylitol was the most profitable product (Mussatto *et al.*, 2013). Another techno-economic analysis of the production of xylitol, ethanol and polyhydroxybutyrate from BSG in Colombia also found xylitol to be the most profitable product, with total cost of production reported as US$ 0.35/kg (A. Dávila, Rosenberg and A. Cardona, 2016), less than half found by the study in Brazil (Mussatto *et al.*, 2013). Neither of these simulated processing facilities extracted BSG proteins from the BSG, which could have improved process economic viability due to the economic value of protein. Both the studies were at large scale of production, i.e. 4800 tonnes per day, which cannot be practically realised considering the production scales of breweries (few produce more than 500 tonnes per day) and the significant limitations to BSG transport (Buffington, 2014). Both studies also found the production process to be heat intensive, since heat integration reduced the cost of production by 40%, which can be attributed largely to the use of BSG with a low dry matter content (11% dry matter) used in the process. The use of such low dry mater content was reported as a hotspot in an environmental impact assessment of XOS production from BSG with the autocatalytic LHW hydrothermal production process applied' s share in the global process impact, ranging between 35% and 55% of all the impacts considered (González-García, Morales and Gullón, 2018). However, by applying more efficient HTT technology, or process intensification, in the valorisation concepts with higher solids concentrations, or reduced water, the impact on the environment (González-García, Morales and Gullón, 2018) and the economics could be improved as reported for other biomass (Larsen *et al.*, 2008; Modenbach and Nokes, 2012; Kolfschoten, Bruins and Sanders, 2014). The economic improvement with process intensification can support viability of a small scale (< 2000 tonne dry biomass per day) biorefinery concept (Kolfschoten, Bruins and Sanders, 2014). Additionally, a small scale biorefinery approach (Bruins and Sanders, 2012), annexed to a brewery can reduce logistics cost, improve water use and lower CO_2 footprint at a brewery (Monin, 2016).

The aim of this study is to investigate whether the process intensification in HTT of BSG (Chapter 4 and 5), namely, use of significantly (>60%) less water and temperatures can make a small scale biorefinery annexed to a brewery, profitable by producing a protein fraction from the BSG, xylitol and/or XOS. To the authors knowledge this is the first study to investigate the profitability of these high value product combinations on a practical scale of BSG availability using the improved HTT technology. To this end, the first objective is to develop and describe three processes for extracting a BSG protein fraction and producing (i) xylitol, (ii) XOS, (iii) both together, effectively assessing XOS production alone with the use of enzymes (i) and

without (iii). The second objective is to determine if a small scale biorefinery scenario provides a viable financial outcome, considering the capital and operational expenditures associated with each scenario. The final objective is to identify key parameters for which the biorefinery profitability is most sensitive.

6.2 Methodology

Three process flow scenarios were developed for small scale biorefinery concepts annexed to a brewery for producing xylitol, XOS and both together from BSG, using improved high solids loading hydrothermal technology from work done in bench-scale stirred reactors (Chapter 4) and pilot scale steam explosion (Chapter 5). Applicable process data was also sourced from literature, including operating conditions, yields and conversion on all downstream processing including XOS production and xylitol fermentation, purification and crystallisation.

6.2.1 Feedstock and logistics

The plant is annexed to a large brewery that provides a feed flow rate of 150 t dry BSG/d. The Table 6-1 show the BSG composition on a dry basis (Chapter 5).

Table 6-1 Composition of feedstock BSG

Component	Mass %	Solid	Flow kg/d
Moisture %	(85.0%)		850
Starch	**4.1**	5.0%	6.2
Cellulose	**12.9**	15.8%	19.4
Hemicellulose			
Xylan	**13.2**	16.1%	19.8
Arabinan	**6.1**	7.4%	9.2
Acetyl group	**1.5**	1.8%	2.3
Lignin	**18.2**	22.2%	27.3
Protein	**21.5**	26.3%	32.3
Ash	**4.4**	5.4%	6.6
Extractives/Lipids	**18.1**		27.2
Total	**100**		**1000**

6.2.2 Development of process flowsheets and simulations

6.2.2.1 Process description

The small scale biorefinery scenarios studied in this work are annexed to a large brewery and produces a variety of products from the brewery BSG waste stream. Hydrothermal processing is applied to BSG after screw-press dewatering, to extract mainly the hemicellulose fraction of the residual lignocellulosic fibre, while producing a steam treated cellulose-lignin (cellulignin) residue possibly more digestible (Kemppainen *et al.*, 2016) and higher-valued animal feed

(Tucker *et al.*, 2004) compared to raw BSG. Xylitol and XOS are primarily produced utilising the xylan extracted from hemicelluloses in the feedstock BSG, during the HTT processing step. The proposed process scenarios are shown in the block flow diagram in Figure 6-1. Primarily xylitol is produced in Scenario A, XOS in Scenario B, while a Scenario C includes co-production of xylitol and XOS products. Additional co-products from all the scenarios include brewers dried protein (BDP) and a brewers condensed solubles (BCS). Process steam and electricity are purchased and residues including cellulignin solid as animal feed.

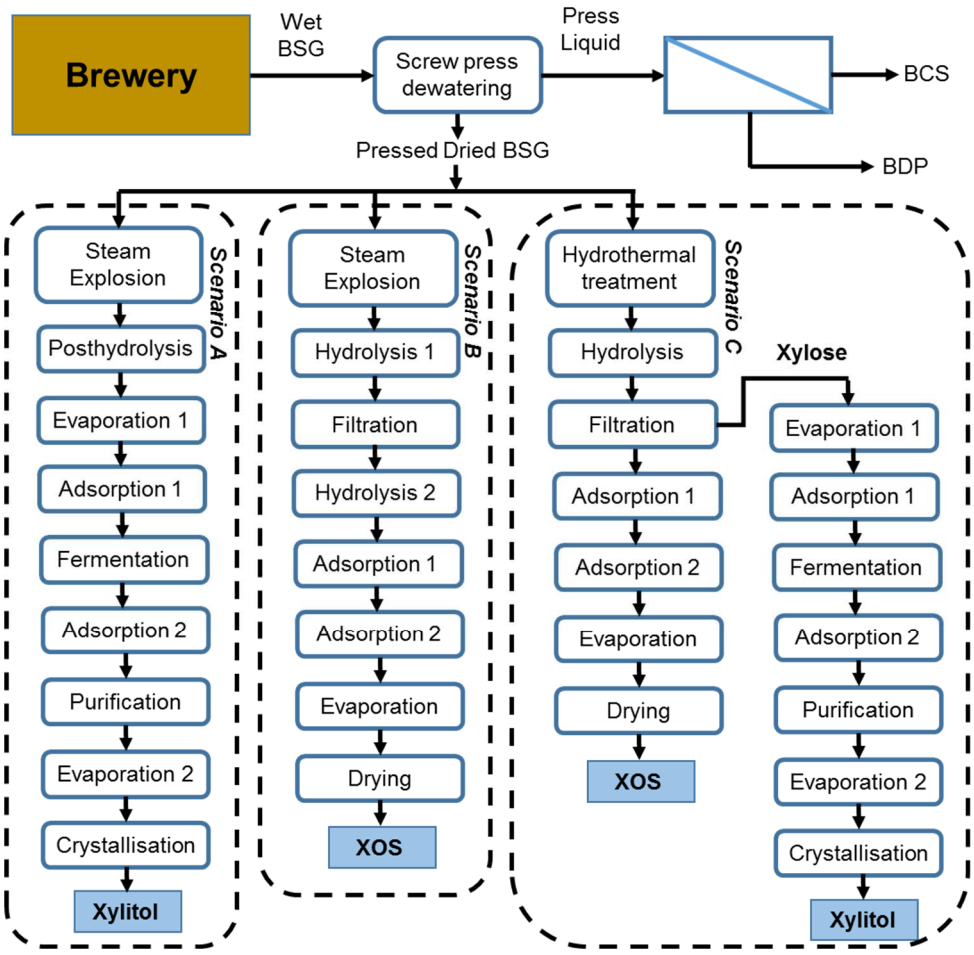


Figure 6-1 Simplified block flow diagram for the main processes of the three scenarios considered: *Scenario A* – Xylitol; *Scenario B* – XOS and *Scenario C* – Xylitol and XOS

6.2.2.2 Descriptions of Scenarios processes

1. Scenario A - Xylitol production

In a first step, prior to the hydrothermal process, raw BSG received from the brewery (85% moisture and 60 ºC) is dewatered using a screw press to extract a large part of the BSG protein and increase the fibre fraction in the pressed BSG (Huige, 1994; Schwencke, 2006). A centrifuge is used to separate the suspended solids from the press liquid (Finley, Walker and Hautala, 1976; Stiles and Herbert, 1977) leaving a clear liquid that is concentrated to 20%

TDS in multiple effect evaporators to brewers condensed solubles (BCS) (Huige, 1994). The recovered suspended solid fraction, high in BSG protein (>30%), is dried to 8 wt.% moisture content in a spray dyer (Ibbett *et al.*, 2019). On the other hand, the pressed BSG is transferred for hydrothermal processing in a high solids screw reactor (Humbird *et al.*, 2011) and is treated with saturated steam at 180 ºC for 10 min (1:3 solid to liquid ratio) where it is assumed 74.2% of the BSG xylan is extracted in the hydrolysate as XOS oligomers (Table 6-2). The process conditions and yields are reasonable to assume for screw reactor HTT of BSG (Lischeske *et al.*, 2016) since they were confirmed in bench scale stirred reactors (Chapter 4) and pilot scale steam explosion reactors (Chapter 5). The hydrolysate is separated from the insoluble residue, cellulignin, by centrifuge at 80 ºC to minimise risk of precipitation of XOS (Gray, Converse and Wyman, 2007). Afterwards the cellulignin is washed using ratio of 2:1 water in a washing step to minimise oligomer loss to <5% (Nieder-Heitmann, Haigh and Görgens, 2018). The hydrolysate is pumped to a pressurised stirred reactor for acid post-hydrolysis at 125 ºC using 0.5 wt.% H_2SO_4 for 165 min (the equivalent of combined severity factor of 1.1) to produce xylose by depolymerisation of the XOS oligomers (Vigo-ourense and Lagoas, 2004; Rivas *et al.*, 2009). In preparation for fermentation, the hydrolysate is concentrated through evaporation (70 ºC) to a xylose concentration of 70 g/l, where volatile components are removed (Carvalheiro *et al.*, 2005). This is followed by neutralisation of the hydrolysate pH to 6.5 through the addition of lime; and the precipitated gypsum and other impurities are removed by filtration (Mussatto and Roberto, 2008). The hydrolysate is further purified by adsorption using activated carbon (3.5 wt.%) to further reduce fermentation inhibitors (Rivas *et al.*, 2009; Vallejos *et al.*, 2015). A final concentration is done of the xylose to 100 g/l by evaporation to further remove volatiles and serves as sterilisation of the hydrolysate for fermentation.

Fermentation of the neutralised xylose hydrolysate is carried out in a batch stirred fermenter under micro-aerobic conditions using *D. hansenii NRRL Y-7426,* a non-pathogenic halotolerant yeast found in cheese and cured meats production (Prista *et al.*, 2016); which as non-GMO, presents no hazard to the environment (Margulis, 2006). Fermentation was conducted with a microbial loading of 12.5 g/l at pH 6.5 at 30 ºC for 60 h (0.81g xylitol/g xylose) that resulted in the yeast fully consuming glucose with minimal arabinose assimilation (Parajó, Dominguez and Domínguez, 1997; Rivas *et al.*, 2009). After fermentation is completed the yeast, soluble salts and organic impurities need to be removed to improve the purity of the xylitol broth (Wei *et al.*, 2010; Martínez *et al.*, 2015).

Yeast and suspended particles are removed through a strainer and finer particles by centrifugation. Separation of soluble compounds are done by adsorption in two stages to reach a xylitol purity >60% of TDS (Heikkila *et al.*, 1992; Zhang, 2002). Firstly colloids, phenolics and colouring organic compounds are removed with adsorption on activated carbon (4% (w/v)

at 60 ºC for 50 min), while salts and inorganics are removed with a combination of two ion exchange resins in series (Carvalheiro *et al.*, 2005; Wei *et al.*, 2010) where after the broth is concentrated at 70 ºC by evaporation to 45% dm. A final purification stage, before crystallisation, increase the xylitol purity >95% by chromatographic separation in a simulated moving bed (SMB) using Amerlite CR1320-Ca resin at 60 ºC with water as mobile phase with a 5:1 ration to xylitol feed (Heikkila *et al.*, 1992; Zhang, 2002). A fraction separated high in arabinose (>50% of TDS) is recovered and concentrated by evaporation to 60% dm (400 g/l arabinose) as a co-product (Zhang, 2002).

The purified xylitol fraction is concentrated to near saturation (650 g/l at 50 ºC) in multiple effect evaporators before proceeding to crystallisation. Xylitol is crystallised in a two stage crystallisation from the saturated solution at 50 ºC after seeding with 1 g/l fine xylitol crystals and cooling to -10 ºC over 48 h (De Faveri *et al.*, 2002; Sampaio *et al.*, 2006). Xylitol crystals with 99% purity are separated by centrifuge from the spent mother liquid containing *circa* 50% TDS other sugars and impurities. The crystals are washed and dried to produce xylitol crystals. Spent mother liquid and wash water are recycled back to purification (Da Silva and Chandel, 2012).

2. Scenario B - XOS production

Similar to Scenario A, the raw BSG received is dewatered mechanically by a screw press and the pressed BSG is treated hydrothermally at the same autocatalytic hydrothermal process conditions (Table 6-2) to fractionate the BSG hemicellulose xylan. The hydrolysate is pumped directly for XOS production, purification and drying (Gomez *et al.*, 2015). Firstly, the hydrolysate is treated with amylo-glucosidase in a stirred batch reactor at 50 ºC for 30 min at pH 4.0 to depolymerise soluble maltodextrins to glucose to enable their removal in the ultrafiltration (Misailidis *et al.*, 2009; Wilkinson *et al.*, 2016). A constant volume (CV) diafiltration step (25 ºC using a 1 kDa molecular weight cut-off membrane and 3 bar trans-membrane pressure) where, the hydrolysate was diluted with water (ratio 2:1 of water to hydrolysate) and in the process concentrated back to its original volume was performed. By repeating the process twice, the concentration of monosaccharides and other soluble non-volatile non-sugars reduced by 72% and 21% respectively, while 83.6% for XOS is recovered (Gomez *et al.*, 2015).

After the diafiltration step, to selectively increase the fraction of short chain XOS, a partial enzymatic hydrolysis treatment is performed with endoxylanase (150 XU/kg of liquor) at 40 ºC for 48 h (Gomez *et al.*, 2015). Further purification of the treated hydrolysate increased (XOS + ArOS) purity to >80% of TDS, by an adsorption step using a strong-anion-exchange resin (Amberlite IRA 400) and an acidic cation-exchange resin (Amberlite 200) in serie, each for

24 h at 25 ºC using a liquor/resin mass ratio of 10:1 g/g (Amberlite IRA 40) and 15:1 g/g Amberlite 200 (Gomez *et al.*, 2015). Drying of the product is achieved in two stages, first concentration to 15% dm by evaporation in multiple effect falling film evaporators (<60 ºC) while spray drying reduces the moisture to <8% in the final XOS product.

3. Scenario C - XOS and xylitol production

Similar to scenario A and B, the BSG received is dewatered mechanically by a screw press and the pressed BSG is treated hydrothermally at 120 ºC for 15 min using 46 mg H_2SO_4 acid/g dry BSG that results in 47.2% XOS and 37.4% xylose yield (Table 6-2). After separating the hydrolysate from the residual insoluble cellulignin, the hydrolysate is treated with lime to increase the pH to 4.5 and precipitated impurities and gypsum are removed by a drum filter. The remaining GlcOS impurities are hydrolysed with amyloglucosidase to glucose to enable their removal in a subsequent membrane filtration step similar to Scenario B. On the other hand, to remove the glucose and other monomeric sugars in the hydrolysate, and thereby to purify the XOS, two sequential steps of membrane filtration were used; firstly a concentration by volume reduction (VR) and secondly constant volume (CV) diafiltration. The CV diafiltration step is applied similar to scenario B, yet prior, a volume reduction (VR) filtration step reduced the hydrolysate volume by 28% while retaining >99% of the XOS (Gómez *et al.*, 2013; Gomez *et al.*, 2015). The filtrates streams from both VR and CV filtration stages are combined and sent for concentration (75% reduction in volume) by nanofiltration (300 Da MWCO polyamide membrane, 20 Bar 40 ºC at 24 l/m^3h); yet before xylitol fermentation, a final concentration by evaporation to 100 g xylose/l is performed (Murthy *et al.*, 2005; Ajao *et al.*, 2015). Fermentation, downstream purification and crystallisation follows the same process steps as in scenario A for xylitol production. The XOS stream, retentate from diafiltration, is sent for purification adsorption process step using a strong-anion-exchange resin and an acidic cation-exchange resin sequentially, as per scenario B for XOS production.

6.2.2.3 Approach to process simulation and assumptions

The conceptualised biorefineries were simulated in Aspen Plus® version.8.8 (AspenTech, Cambridge, MA), process simulator to determine mass and energy balances and calculate requirements for utilities including electricity, cooling water, air and steam. Due to the varied composition of streams and presence of electrolites the Electrolyte Non-Random Two-Liquid (ELECNRTL) thermodynamic property method was used as base model to determine state parameters for all process equipment (Gerbrandt, 2014). Component definitions were used from ASPEN property database and NREL component definitions to describe conventional chemicals and unconventional lignocellulose compounds used in the simulations (Wooley and Putsche, 1996; Humbird *et al.*, 2011). Built in Aspen Plus® software packages, Aspen Energy

Analyser was used for utility minimization, and Aspen Process Economic Analyser for equipment sizing, costing and economic analysis (Hegy *et al.*, 2013).

Various calculator and design specification blocks were used in the ASPEN process simulation, to facilitate and automate the process flowsheet calculations (Petersen, Aneke and Görgens, 2014). All evaporators and dryers were modelled as flash drums. Reactors for autocatalytic HTT and enzymatic hydrolysis in XOS production, as well as fermenters were modelled with stoichiometric reactor blocks (Humbird *et al.*, 2011; Mandegari, Farzad and Görgens, 2016). Dewatering equipment were modelled with centrifuge blocks with a 5% sugar loss with solids; except for the BSG screw press, where a separator block was used with specified separation of components to press liquid and pressed solid streams. Separator blocks were also used for ultra- and nanofiltration units (Gómez *et al.*, 2013; Gomez *et al.*, 2015), separation of xylitol in SMB chromatographic separation (Heikkila *et al.*, 1992; Zhang, 2002) and for purification of the xylitol-containing fermentation broth, the activated carbon and ion exchange resin columns according to reported data (Wei *et al.*, 2010). In XOS production separator blocks were used for nanofiltration of the HTT hydrolysate and resin adsorption columns used for XOS purification according to reported data (Gomez *et al.*, 2015).

Further process assumptions include:

1. Literature data used for purification and fermentation will be applicable to an industrial process to make the results from study valid for the level of accuracy required.
2. 10% loss of energy to environment on heat integration between processes modelled in Aspen Plus®.
3. The base case considered assume a value of US$10/t of wet BSG in the current use as animal feed supplement (Schwencke, 2006; Rosa and Beloborodko, 2015; Kerby and Vriesekoop, 2017).
4. Reported processes for acid post hydrolysis, XOS purification and enzymatic hydrolysis using low dry matter content can be used in simulations with more appropriate increased dry matter content (Franceschin *et al.*, 2011; Clauser *et al.*, 2015; Nieder-Heitmann, Haigh and Görgens, 2018).
5. After hydrothermal treatment, the balance of the yield of carbohydrates in the hydrolysate, as sugars and inhibitors, was assumed remaining in the insoluble cellulignin.
6. While more conservative assumptions for the protein and lignin are assumed that takes all mass lost in HTT from the residual cellulignin solids to be hydrolysed instead.
7. Experimental hydrothermal process conditions:

Results from the study of the extraction of BSG hemicellulose xylan using bench scale stirred batch (chapter 4) and pilot scale steam explosion HTT (Chapter 5) established applicable

process condition that were applied for the process scenario's (Table 6-2). A preferred autocatalytic HTT process condition at 180 ºC and 10 min (80.3% total xylan recovery and 74.2% XOS yield) was used for scenario A and B, while for scenario C, results from LHW HTT in stirred batch system using acid dosing of 46 mg H_2SO_4/g dry BSG at 120 ºC for 15 min (85% total xylan recovery and 47% XOS yield). Glucose, GlcOS (gluco-oligosaccharide including malotose and soluble starch) and 5-hydroxymethylfurfural (HMF) are reported based on the initial starch and residual insoluble solid content showed the extent of protein, lignin and ash reduction in the HTT process that was used. The dry matter to water ratio of 1:3 was used for HTT in all scenarios according to the experiments, even though in other techno-economic assessments of lignocellulosic biorefineries further increased dry matter content was simulated to achieve more possible energy savings (Franceschin *et al.*, 2011; Clauser *et al.*, 2015; Nieder-Heitmann, Haigh and Görgens, 2018).

Table 6-2 Hydrothermal treatment of BSG: Yield of sugars, inhibitors and dissolved solids

Components	Steam explosion[a] (Scenario A and B)	LHW Batch[b] (Scenario C)	Basis
	180 ºC 10 min	120 ºC 15 min 46 mg/g dry BSG	
Sugars and inhibitor yield in hydrolysate			
GlcOS	101.5%	80.6%	Starch
XOS	74.2%	47.2%	Xylan
ArOS	38.2%	20.5%	Arabinan
Glucose	3.7%	16.7%	Starch
Xylose	6.1%	37.4%	Xylan
Arabinose	33.8%	78.8%	Arabinan
Acetic acid	47.0%	40.4%	Acetyl
Formic acid	0.9%	0.7%	Xylan
HMF	1.5%	0.6%	Starch
Furfural	2.0%	2.8%	Xylan
TDS ND[c] (g/100 g dry BSG)	9.96	9.17	
Reduction in compound per residual insoluble solids[d]			
Protein	45.0%	68.5%	
Lignin	42.6%	31.5%	
Ash	58.8%	51.0%	

a Results are average from two types of BSG (Chapter 5)
b Results from Chapter 4
c TDS content not determined in the resulting hydrolysate
d Results from insoluble residue obtained after HTT

6.2.3 Economic assessment

The economic analysis aims to determine the net financial benefit of a small scale biorefinery annexed to a brewery utilising spent brewery waste BSG for mainly xylitol or XOS production. The equipment costs and operating costs are assessed to determine if a scenario provides a viable economic business compared to the current disposal as animal feed. Results are presented in terms of 2018 US dollars.

6.2.3.1 Capital and operating costs

i. Capital expenditure (CAPEX) estimation

Purchased and installed cost of equipment used in the process scenarios were determined using the Aspen Plus Economic Analyser®. For reactors, pressure vessels, SMB systems and membrane filtration units purchase prices from literature were used. Costs were adjusted for capacity and for assessment time by using 603.1 for the Chemical Engineering Plant Cost Index on 2018 basis (Towler and Sinnott, 2008; Humbird *et al.*, 2011). Using all the installed costs, the inside-battery-limits (ISBL) cost is determined as the sum of costs involved in production excluding utilities, storage, and waste water. The total direct cost (TDC) for the new biorefinery was determined as sum of total installed cost (TIC) including utilities (6.5% of ISBL), storage and warehouses (9% of ISBL), site development (9% of ISBL) and additional piping cost (4.5% of ISBL). Using TDC, total indirect cost (TIC) is estimated as sum of field expenses, contingencies, pro-rateable costs, and other costs each rated as 10% of TDC, together with office construction cost as 20% of TDC. The fixed capital investment (FCI) is calculated as sum of total direct and indirect costs. A 5% working capital (WC) is assumed and WC together with FCI provides an estimate of total capital investment (TCI) (Assessment, Selected and Gorgens, 2016). After calculating the CAPEX it is prudent to calculate the OPEX.

ii. Operating expenditure (OPEX) estimation

Operating expenditure consists of variable and fixed operating costs in the production pathway of a product such as xylitol or XOS. Fixed operational expenses include costs that do not vary with production and are fixed for the plant such as overheads, property taxes, insurance, plant maintenance and labour or salaries. Estimates for property taxes and insurance are 3% of ISBL and maintenance 0.7% of TCI, while for labour, rates from the ethanol industry were used (Assessment, Selected and Gorgens, 2016; Ali Mandegari, Farzad and Görgens, 2017). Variable operating cost vary with production scale or with change in units produced and are calculated from the energy and mass stream flow rates in the process simulation. The variable costs consists of steam, electricity, waste streams, labour and raw materials costs, which include feedstock, consumable chemicals, and reagents (Table 6-3). Additional capital charges can include periodic charges for replacement items in production such as ion exchange resin, filtration membranes or activated carbon units. Total cost of production (TCP) includes operating expenses and general expenses such as depreciation and interest (Table 6-3).

Table 6-3 Costs of raw materials and utilities

Feedstock		
BSG (85% moisture)[a]	10	$/t
Reagents		
H_2SO_4[b]	50	$/t
Lime[b]	120	$/t
Oxygen[b]	300	$/t
Amyloglucosidase[b]	3	$/kg
Endoxylanase[b]	8	$/kg
Utilities		
Water[c]	1.2	$/m3
Electricity[c]	0.2	$/kWh
Steam LP[c]	3	$/t
Steam MP[c]	8	$/t
Cooling water[c]	0.02	$/t
Chilled water[c]	0.15	$/kWh
Wastes		
Waste water[c]	0.8	$/m3
Gypsum[c]	7.5	$/t
Periodic capital items		
Activated carbon (2 years)[d]	0.9	$/kg
IE resin (5 yeasrs)[e]	4.7	$/kg
Regenerating AC[f]	0.020	$/t
Regenerating IE resin[f]	0.196	$/t

a Estimate of transport cost within 50 km
b Average from different commercial sites and literature (Mussatto *et al.*, 2013; Hong *et al.*, 2014; Clauser *et al.*, 2015; Petersen, Franco and Görgens, 2018)
c Averages from reported literature (Towler and Sinnott, 2008; Mussatto *et al.*, 2013; Hong *et al.*, 2014; Clauser *et al.*, 2015; Petersen, Franco and Görgens, 2018)
d (Mussatto *et al.*, 2013)
e (Wooley, 1997; Wan, Xie and Nancy Ho, 2003)
f (Wan, Xie and Nancy Ho, 2003)

6.2.3.2 Discounted cash flow

Calculation of total cost of production (TCP) for a product such as xylitol will give an estimate of breakeven cost for xylitol. If market prices for xylitol are lower than TCP and there are no other revenues from production the biorefinery concept will be unprofitable. Yet the economic analysis is done by assessing net present value (NPV), internal rate of return (IRR) and minimum required selling price (MRSP), by using projected cash-flow estimates for the proposed scenarios simulations. Capital and operating expenditures were used together in a real term discounted cash flow (DCF) analysis against revenues derived from sales using assumed market-based selling prices for xylitol and XOS. The average selling prices for products were estimated from prices quoted in international markets and literature (Table 6-4). The DCF assume operating costs and revenues remain constant over the project's lifespan. The IRR for each scenario can be assessed against expected return of capital invested, hurdle rate of 9.7% (Assessment, Selected and Gorgens, 2016; Nieder-Heitmann, Haigh and Görgens, 2018). Alternatively, the economic analysis can calculate the minimum required

selling prices for xylitol and XOS in each of the process scenarios. The MRSP is the minimum selling prices of products that would generate sufficient cash flow to achieve an IRR equal to the weighted average cost of capital (WACC) or hurdle rate, just enough to break even. An economic sensitivity analysis is used to identify the important parameters on viability and profitability of the various scenarios.

Table 6-4 Economic parameters

Financial model assumptions	
Working capital (% of FCI)[a]	5%
Project life (Years)[a]	25
Discount rate (real) [a]	9.7%
Depreciation method (5 years)[a]	Straight line
Salvage value[a]	0
% Spent in year 0[a]	100%
Start-up time (Years)[a]	0
Operating hours per year[a]	8000
Income tax rate[a]	28%
Cost year for analysis[a]	2018
Inflation rate[a]	5.7%
Products values ($/kg)	
Xylitol[b]	4.5
XOS[c]	4.5
Co-products values ($/t)	
Cellulignin[d]	8
Brewers dried protein (BDP)[e]	350
Brewers condensed solubles (BCS)[f]	10
Yeast[g]	150
Arabinose syrup[h]	400

a (Assessment, Selected and Gorgens, 2016; Petersen, Franco and Görgens, 2018)
b (Gerbrandt, 2014; Biddy *et al.*, 2016)
c (Llzo and Lx, no date)
d Considered improved or similar feed value as BSG(Tucker *et al.*, 2004)
e Based on protein content using $1/kg protein
f Price of molasses
g Based on protein content wet basis
h (Michael O'Donohue, 2014; Clauser *et al.*, 2015)

6.3 Results

6.3.1 Technical outcomes

The process simulations derived models for the utilisation of BSG in three biorefinery scenario concepts to produce xylitol only (Scenario A), XOS only (Scenario B) and a combination of the two (Scenario C). All three scenarios utilised the same screw press BSG dewatering step prior to the HTT. The HTT for scenario A and B utilised a process at 180 ºC (Chapter 5) that is conducted in a continuous screw type reactor (Humbird *et al.*, 2011). Yet for the Scenario C, a set of stirred batch digesters were used instead for the HTT process and the process operated at lower (120 ºC) temperatures (Chapter 4). The performance from the two types of HTT can be seen from the product recoveries achieved. Using the steam based autocatalytic

hydrothermal process (Chapter 5), in Scenario A and B, 80% of the xylan was recovered, mostly as oligomers in the hydrolysate (Figure 6-2 A). While in Scenario C, 85% xylan recovery was achieved yet with significantly higher monomeric xylose and arabinose sugar (Figure 6-2 B). As a result of the conservative assumption on recovery of lignin and proteinaceous compounds, results show both hydrolysates contain *circa* 50% inhibitors and impurities, higher than the *circa* 40% reportedly found experimentally (Gomez *et al.*, 2015).Therefore downstream purification requirements simulated are conservative.

Scenario C, producing both xylitol and XOS shared all the downstream processing steps from Scenario A and B with only small differences in the filtration process as a result of added nanofiltration units for recovering monomeric sugars separated from the oligomers. Although most process steps can have high yields of >80% (Table 6-2), the solid liquid and product separation involve losses that compound to result in only 37% xylan equivalent recovery in the Scenario A for xylitol production (Appendix Table 6A-20). A comparison and complete breakdown of all process steps and recoveries for the scenarios is given in Appendix Section 6.5. The xylitol production recovery of 37% obtained (Table 6-5) in scenario A is in agreement with other reported studies of xylitol production using autocatalytic hydrothermal treatments (Franceschin *et al.*, 2011; Gerbrandt, 2014; Clauser *et al.*, 2015). The xylan recoveries reported range between 13% to 34% when applying various detoxification strategies for wood chip autocatalytic hydrothermal hydrolysate (Gerbrandt, 2014), 39% from bagasse (Clauser *et al.*, 2015) and as high as 43% in xylitol production from rye straw (Franceschin *et al.*, 2011). Only studies for xylitol production using dilute acid hydrothermal processes reported higher xylan recoveries (>80%) (Mussatto *et al.*, 2013; A. Dávila, Rosenberg and A. Cardona, 2016), that show the need to improve autocatalytic hydrothermal technologies and reduce process steps. The reduced number of process steps, such for XOS production in process scenario B, achieved 52% overall xylan yield and 22% arabinan in the XOS product (Table 6-5). Yet the combined XOS and xylitol production in Scenario C reached 55% xylan equivalent recovery (18% as xylose and 37% as XOS). This is partly as a result of the higher recovery with the acid used in HTT and the simultaneous recovery of XOS oligomers and monomeric sugar xylose as products.

All three scenarios utilised the same BSG dewatering step and produced similar by-products, i.e. BDP and BCS (Table 6-5). Arabinose sugar was also recovered in Scenario A and C as an important by-product from the acid catalyst applied in hydrothermal processing. Additionally, in scenario C, 7% less cellulignin remained compared to other scenarios as a result of the acid dosing with the different HTT used (Table 6-5). Acid use in Scenario A, of 0.5% wt. in the post-hydrolysis was half that of 46 mg/g dry BSG acid dosing used in HTT in Scenario C. This required more lime for neutralisation. Yet, the other HTT used in scenario A

and B, resulted in the highest electricity demand as a result of the high pressure continuous screw reactor system needed. For scenario A and B the reactor system accounted for 71% and 72% of the total electricity demand respectively (Appendix Section 6.7). The heat requirements of the HTT steps were overall low compared to other process units. The single highest heat requirement for all scenarios was the energy required for upgrade of the press liquid to BDP and BCS through evaporation and drying (8378 kW). This consisted of nearly 40% of the overall heating demand in Scenario B and ca 30% for Scenario A and C (Appendix Section 6.7). With heat integration Scenario B resulted with the highest heat requirements yet with the advantage of the lowest cooling water requirement (Table 6-5). Additionally, the XOS production in the scenario B showed the highest water consumption, more than double from other scenarios as a result of the high clean water requirements for the constant volume diafiltration and adsorption resin employed in the purification of the XOS hydrolysate. Scenario C show nearly 50% less water was used while producing nearly 70% the amount of XOS as in Scenario B, as a result of a reduction in volume and impurities with the additional purification by membranes separating the monomeric sugars from the hydrolysate.

The experimental data for the screw press dewatering and hydrothermal treatments sufficiently complimented the literature data used for improved process flow designs simulated in ASPEN Plus®. Overall xylan yield scenario A (37%) in the xylitol production is comparable to other reported autocatalytic hydrothermal studies for xylitol production by fermentation. Results for the XOS production are novel, yet relate well to the xylitol process, therefore the results could be used to determine production costs for an economic assessment.

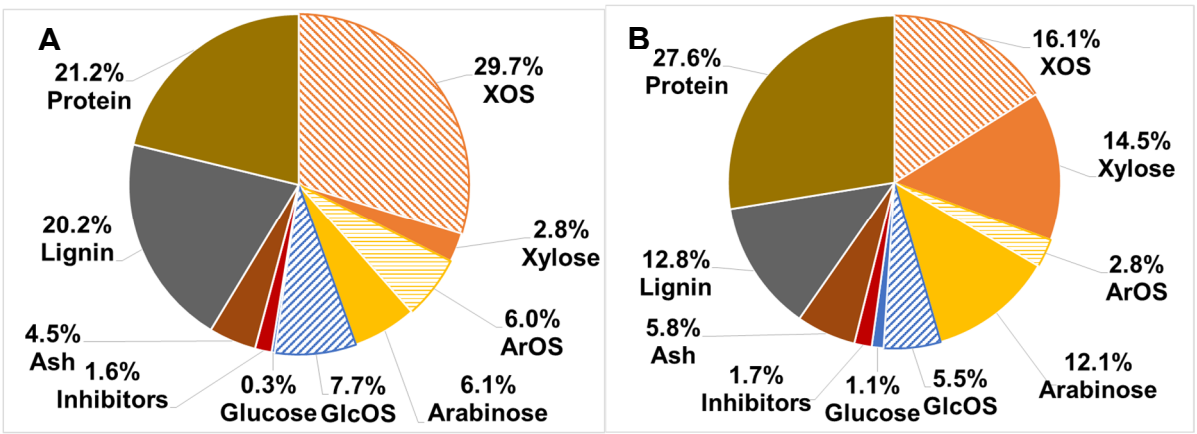


Figure 6-2 Hydrolysate composition results from hydrothermal treatment: (A) Scenario A and B from autocatalytic steam explosion at 180 ºC and 10 min and (B) Scenario C using acid dosed LHW HTT at 120 ºC 46 mg H_2SO_4 15 min (Inhibitors include acetic acid, furfural and 5-hydroxymethylfurfural (HMF))

Table 6-5 Mass and energy balance results for the three scenarios

Scenario		A	B	C	dm%
	Units	Xylitol	XOS	Xylitol & XOS	
Mass balance					
BSG feed rate	kg/h	41667	41667	41667	15.0%
Products					
Xylitol	kg/h	343	-	146	99.9%
XOS[a]	kg/h	-	696	480	92.7%
Overall xylan yield	%	37	52	18/37	
Co-products					
BDP	kg/h	1345	1345	1345	92.0%
BCS	kg/h	1119	1119	1119	20.0%
Cellulignin	kg/h	4450	4450	4125	65.0%
Spent yeast	kg/h	189	-	72	85.0%
Arabinose syrup	kg/h	430	-	399	40.0%
Molasses	kg/h	8	-	4	20.0%
Reagent					
Enzyme A	kg/h	-	2	2	
Enzyme X	kg/h	-	14		
Yeast	kg/h	116	-	38	
H_2SO_4	kg/h	114	-	224	
O_2	kg/h	63	-	29	
Lime	kg/h	89	-	171	
Water	kg/h	39895	102280	48993	
Waste streams					
Gypsum	kg/h	465	-	524	
Wastewater	kg/h	73646	146090	78104	
Regenerating stream					
Activated carbon	kg/h	527	-	99	
IE resin	kg/h	19858	24824	10955	
Energy balance					
Utilities					
LP Steam[b]	kW	11103	16750	14011	
MP Steam[c]	kW	201	201	0	
Cooling water	kW	32898	19428	28524	
Chilled water	kW	350	0	315	
Electricity	kW	855	836	313	

a XOS product mass include ArOS
b Steam at 233 °C and 9.5 atm
c Steam at 266 °C and 13 atm

6.3.2 Economic outcomes

6.3.2.1 Capital expense (CAPEX)

The energy and mass balances derived from ASPEN Plus® simulation of the processes (Table 6-5) were used subsequently to estimate the size of equipment required, installed and operational costs. The resulting total capital investment (TCI) for the scenarios are shown in Table 6-6. Scenario B for XOS production process resulted in the most capital intensive scenario of the three. Process sections for XOS purification and the autocatalytic HTT sections

in Scenarios A and B are the most capital intensive with *circa* MM$5-6 each. Both scenario A and B applied a continuous screw type reactor in HTT that consisted of 71% and 78% of the total cost of the section respectively (Appendix Section 6.8). Purification of XOS consists of adsorption steps through three large (200 m^3) fixed bed resin vessels that contributed each >MM$1 to installed capital expense. Although there are no capital estimates to compare XOS production, for xylitol production in scenario A the estimated FCI per tonne xylitol product capacity per day (8.4t/d), MM$4.26/t, is comparable to a reported value of MM$4.53/t for xylitol production by fermentation at a similar scale (9t/d) from rye straw using autocatalytic HTT (Franceschin *et al.*, 2011).

Compared to Scenario A and B, Scenario C showed the best economic benefit with the highest NPV (MM$78) and lowest minimum required selling price (MRSP) of US$2182/t for the xylitol and XOS products (Table 6-6). This MRSP in Scenario C is nearly half that is required for Scenario A with production of xylitol alone (US$4153/t). Moreover, excluding any sales revenue from xylitol in Scenario C resulted in a 30% increase in MRSP for XOS to US$2847/t and just 13% higher than Scenario B for XOS production alone (Table 6-6). The lower TCI from scenario C, mainly from the HTT process section, using stirred batch reactor for LHW, and the overall higher xylan recovery contributed to improved profitability compared to scenario A and B. However the by-product revenue and operating costs also favourably supported the economics.

6.3.2.2 Operating expense (OPEX) and total cost of production (TCP)

OPEX for scenario C was the lowest, MM$12.01/y (Table 6-6). A breakdown of the OPEX finds raw material cost to be the single biggest cost contributor, and larger than annualised cost of capital (ACC) (Table 6-6). Raw material costs are made up of primarily the BSG feedstock cost that consisted of 75%, 61% and 80% for scenario A, B and C respectively (Appendix Section 6.6). The assumed cost of US$10/t wet BSG is the best case scenario for a brewery, many breweries do not have a market for BSG (Schwencke, 2006; Kerby and Vriesekoop, 2017). The reduced share of BSG feedstock cost in scenario B is partly as a result of the cost contribution of the endoxylanase enzyme used for partial depolymerisation of the XOS oligomers. The cost of the endoxylanase used in Scenario B holds certain risk since the cost of these enzymes can be significant. At US$8/kg used in this study, these enzymes alone contributed to 6.1% of the total cost of XOS production in scenario B, the sum of OPEX and ACC. This value is in the range of cost contribution found (6.8%) for xylose production from rye straw (Franceschin *et al.*, 2011).

Total cost of production (TCP) for xylitol (Scenario A) was the highest (US$5.97/t), yet by-product revenue contributed considerably to improve the overall process profitability (Table 6-6). In Scenario A for the xylitol production, 32.7% of the total revenue contribution was sales

from by-products, while only 14.2% for Scenario B and 27.5% for Scenario C (Figure 6-3). The brewers dried protein (BDP) contributed by far the largest portion of by-products revenue for all scenarios (12.9-20.8% of total revenue), followed by arabinose syrup 7.6 and 6.4% of total revenue for Scenario A and C respectively.

Table 6-6 Comparison of capital and operating cost estimates, NPV and MRSP of the scenarios

Capital Expense - CAPEX ($)			
Process scenario	**A**	**B**	**C**
Main product	**Xylitol**	**XOS**	**Xylitol & XOS**
Area A100: Feedstock preparing	$292 258	$292 258	$292 258
Area B100: Press liquid upgrading	$2 207 800	$2 207 800	$2 207 800
Area A200: Hydrothermal treatment	$6 277 568	$5 688 800	$1 749 529
Area A300: Neutralisation			$1 097 824
Area A350: Filtration		$3 072 815	$1 423 108
Area A360: XOS Purification		$4 683 706	$2 396 716
Area A370: XOS Drying	$937 523	$1 680 400	$1 438 800
Area A400: Fermentation	$2 355 540		$1 476 122
Area A500: Xylitol Purification	$3 043 915		$2 025 465
Area A600: Crystallisation	$1 641 500		$1 464 200
Area A700: Wastewater	$257 300	$698 500	$861 500
Total ISBL	**$17 013 406**	**$18 320 952**	**$16 433 322**
Area A800: Storage (5 % of ISBL)	$849 874	$895 663	$820 594
Area A900: Utilities (6.5% of ISBL)	$1 104 836	$1 164 362	$1 066 772
Total Installed Costs (TIC)	**$18 968 114**	**$20 380 977**	**$18 323 154**
Total Direct Costs (TDC)	**$21 945 460**	**$23 587 144**	**$21 198 986**
Total Indirect Costs	**$13 167 276**	**$14 152 286**	**$12 719 391**
Fixed Capital Investment (FCI)	**$35 112 736**	**$37 739 430**	**$33 918 377**
Total Capital Investment (TCI)	**$36 900 000**	**$39 632 969**	**$35 614 296**

Operating Expense - OPEX (MM$/y)				
	Xylitol	XOS	Xylitol	XOS[c]
Production (t/y)	2 748	5 569	1 169	3 840
By-product revenue (MM$/y)	5.75	4.14	5.49	
Variable OPEX (MM$/y)				
Raw Materials	4.43	5.49	1.36	3.03
Waste Streams	0.78	1.40	0.24	0.56
Utilities	3.04	2.43	0.50	1.63
Recurring operating expense	0.05	0.08	0.02	0.02
Fixed OPEX (MM$/y)				
Labour & Supervision	3.59	2.97	0.91	3.00
Other Overhead	0.76	0.81	0.17	0.56
Total OPEX	**12.63**	**13.19**	**3.20**	**8.81**
Annualised cost of capital (ACC)	3.78	4.06	1.60	2.05
Total cost of production[d] ($/kg)	**5.97**	**3.10**	**4.10[e]**	**2.83[e]**
FCI per day tonne (MM$)	$4.26	$2.26	$4.45	$1.74

Discounted cash flow analysis (DCF)				
Nett present value (NPV)	$6 766 777	$75 039 329	$78 465 867	
Minimum required selling price (MRSP)[b]			Single	Combined
Xylitol ($/t)	4153	-	9348	2182
XOS ($/t)	-	2509	2847	2182

a For scenario C cost of process areas shared are split by mass product ratio (23% xylitol contribution)
b MRSP are calculated for one product alone the other $0 and both equally priced
[c] XOS product mass include ArOS
[d] TCP = OPEX+ACC,
[e] TCP in scenario C shared process unit costs split prorate product mass

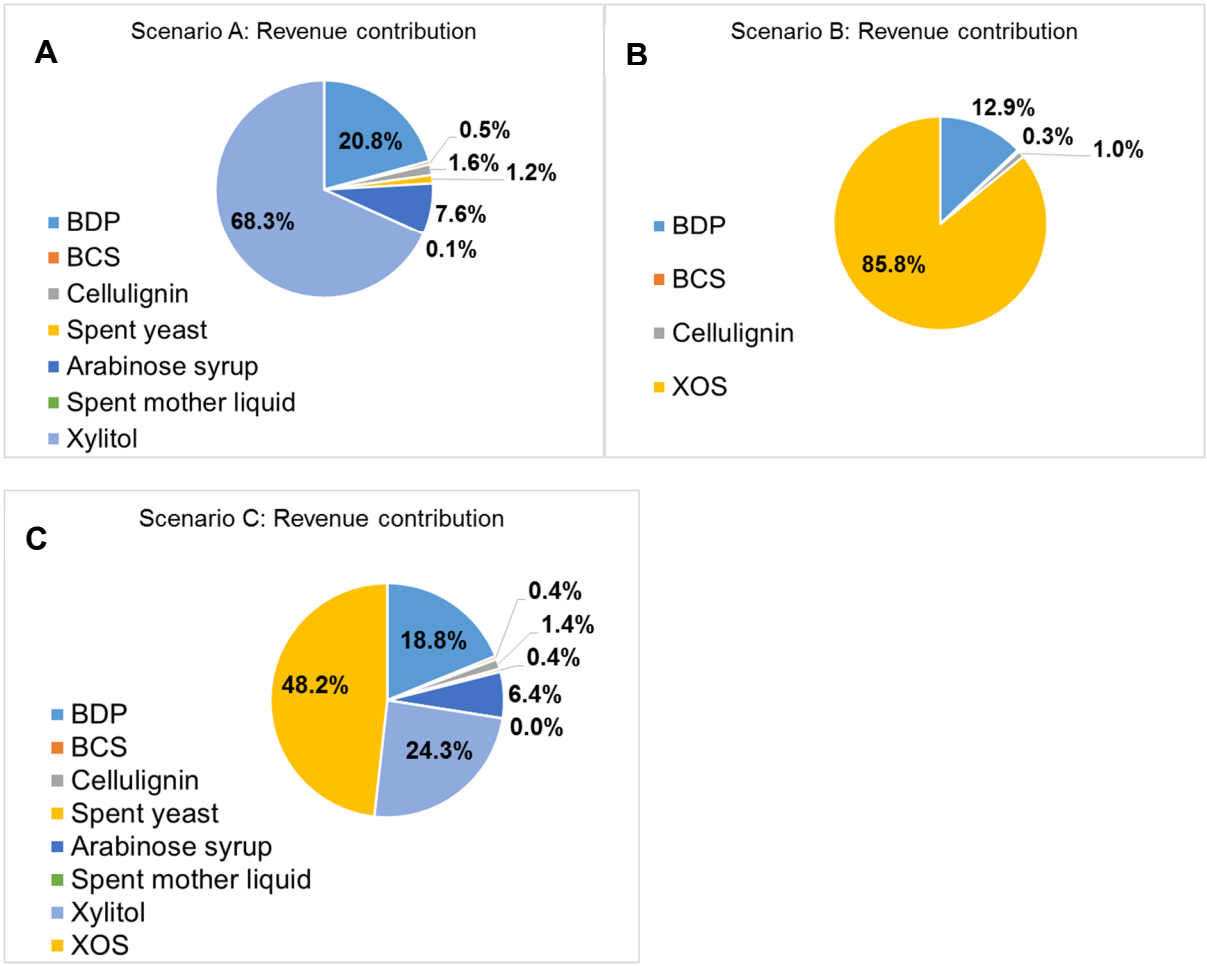


Figure 6-3 Revenue contribution from each product and by-product (XOS includes ArOS)

6.3.2.3 Assessment of the return potential of the scenario's

All scenarios achieved IRR over the 9.7% hurdle rate, with the assumed market price of US$4.5/kg for both xylitol and XOS. However these market prices can fluctuate and to assess the effect on IRR, market values of 2.5 and 6.5 were assessed and their impact on IRR. The resulting IRR are plotted in Figure 6-4. Only Scenario B and C achieved IRR above the hurdle rate at market price of US$2.5/kg. Both Scenario's B and C achieved >50% IRR with market prices of US$6.5/kg, while Scenario C was the highest IRR of 57.9%. This IRR from Scenario C also showed less variance compared to Scenario B (43.3% compared to 44.3%). This is a result of the diversified revenue stream in Scenario C that incorporate also arabinose syrup as by-product; that could be advantageous and support sustainability of a small scale biorefinery.

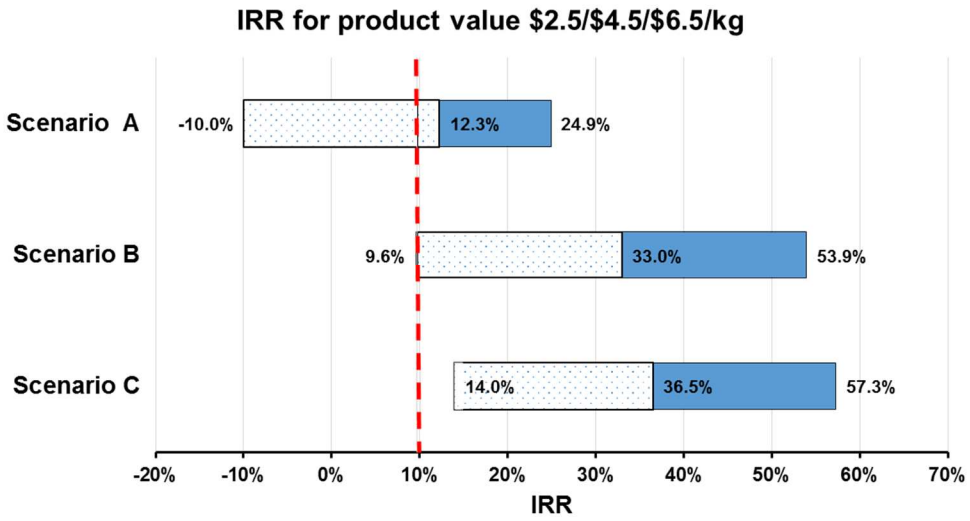


Figure 6-4 Comparison of IRR between the three biorefinery scenarios for product prices ($2.5, 4.5 and 6.5 /kg) (Red line indicate hurdle rate)

The multi-product Scenario C showed by-product revenue contributed to achieve the MRSP of US$2847/t for XOS, for the desired hurdle rate (IRR of 9.7%) with no price for xylitol. Xylitol MRSP as product alone however is much higher at US$9348/t. A correlation of the IRR achieved with the change in xylitol and XOS selling prices show xylitol prices higher than US$5000/t and XOS higher than US$1000/t yield positive IRR (Figure 6-5).

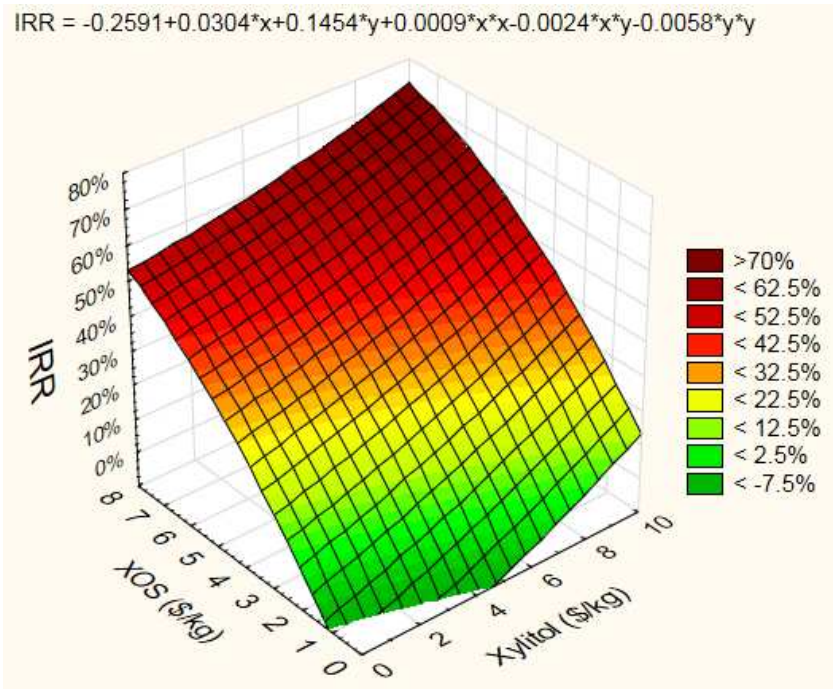
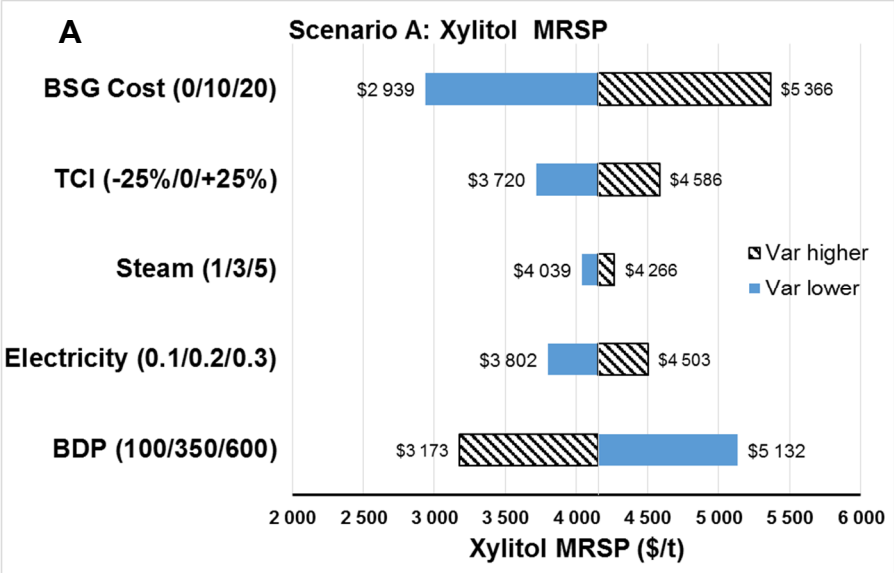


Figure 6-5 Profitability and product process in Scenario C: IRR and xylitol/XOS selling prices

6.3.3 Sensitivity analysis

An economic sensitivity analysis was done to assess important economic parameters on biorefinery scenario profitability. The changes in MRSP for products xylitol and XOS were determined for the three scenarios with changes in feedstock BSG cost price ($0 and $20), TCI, (25% increase and decrease), steam utility cost (US$ 1/t and US$ 5/t), electricity charge (US$ 0.1/Kwh and US$0.3) and by-product revenue BDP (US$100 and US$600/t) as shown in Figure 6-6 A, B and C. The revenue from the selling of BDP is an important part and a decrease in the value from US$350/t to US$100/t increased the MRSP for xylitol to US$5132/t from US$4153/t. Therefore extracting the BSG protein and upgrading the quality of the BDP can result in significant economic impact on the small scale biorefinery. Yet for all three scenarios the BSG cost had the largest effect on MRSP of the xylitol and XOS. Lowering the cost of BSG feedstock to US$0/t had the largest decrease of MRSP in all scenarios with Scenario C reaching the lowest US$1517/t for xylitol and XOS (Figure 6-6 C) and to US$2939/t for xylitol alone in Scenario A. A near zero BSG feedstock cost is not impossible for a small scale biorefinery adjacent to a brewery since many breweries do not have the option to sell their BSG to farmers.



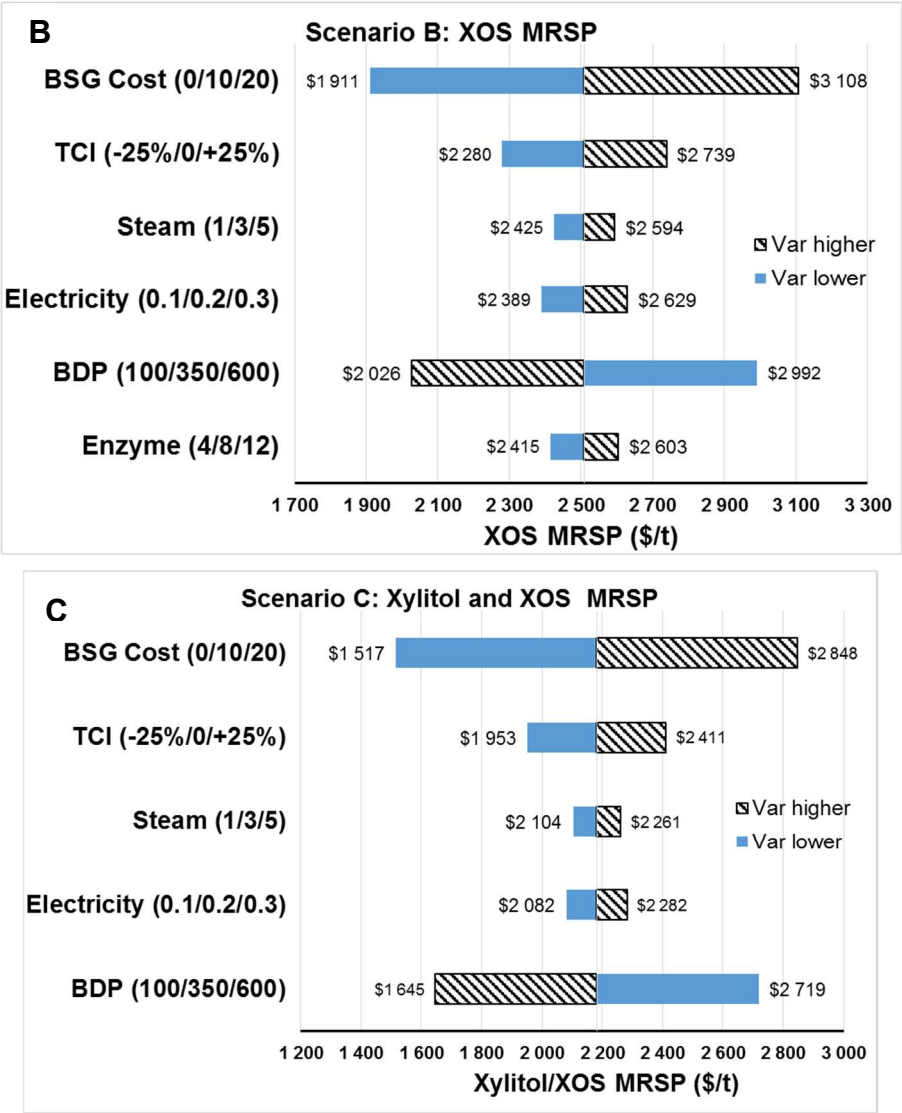


Figure 6-6 Economic sensitivity analysis of variance of parameters (BSG cost, TCI, Steam cost, Electricity cost and by product BDP value: (A) Scenario A, (B) Scenario B and (C) Scenario C.

6.4 Conclusion

A small scale (150 tonnes BSG per day) biorefinery can be profitable by valorizing the BSG produced from a large brewery, annexed to it and by applying high solids HTT technology to produce high value products xylitol and XOS. The production of a single product such as a prebiotic XOS from BSG was found to be economically more favorable compared to the more elaborate xylitol product, with a MRSP for XOS of US$2509/t, nearly half that of xylitol (US$4153/t). Additionally, by using appropriate HTT technology to produce XOS and xylitol together, improved overall product yields that reduced MRSP to US$2182/t for the products. It was shown that the by-product revenue contributions including BSG protein in a multiproduct scenarios support the economic viability of the concept of a small scale biorefinery annexed to a brewery.

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E-supplementary data of this work can be found in online version of the paper.

6.6 References

Ajao, O., Rahni, M., Marinova, M., Chadjaa, H., Savadogo, O., 2015. Retention and flux characteristics of nanofiltration membranes during hemicellulose prehydrolysate concentration. Chem. Eng. J. 260, 605–615. https://doi.org/10.1016/j.cej.2014.09.007

Ali Mandegari, M., Farzad, S., Görgens, J.F., 2017. Economic and environmental assessment of cellulosic ethanol production scenarios annexed to a typical sugar mill. Bioresour. Technol. 224, 314–326. https://doi.org/10.1016/j.biortech.2016.10.074

Ali Mandegari, M., Farzad, S., Görgens, J.F., 2016. Process Design, Flowsheeting, and Simulation of Bioethanol Production from Lignocelluloses, in: Biofuels: Production and Future Perspectives. pp. 261–288.

Biddy, M.J., Scarlata, C., Kinchin, C., 2016. Chemicals from Biomass: A Market Assessment of Bioproducts with Near-Term Potential NREL/TP-5100-65509.

Blinkov, S.D., Nemoytin, J.B.B.M., Fedorov, A.L., 1999. Method for processing vegetal raw materials in order to produce pentose hydrolysates essentially containing xylose. WO1999023260.

Bruins, M.E., Sanders, J.P.M., 2012. Small-scale processing of biomass for biorefinery. Biofuels, Bioprod. Biorefining 6, 135–145. https://doi.org/10.1002/bbb1319

Buffington, J., 2014. The Economic Potential of Brewer's Spent Grain (BSG) as a Biomass Feedstock. Adv. Chem. Eng. Sci. 308–318.

Carvalheiro, F., Duarte, L.C., Lopes, S., Parajo, J.C., Pereira, H., Girio, F.M., 2005. Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by Debaryomyces hansenii CCMI 941. Process Biochem. 40, 1215–1223. https://doi.org/10.1016/j.procbio.2004.04.015

Carvalheiro, F., Esteves, M.P., Parajo, J.C., Pereira, H., Gıırio, F.M., 2004. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91, 93–100. https://doi.org/10.1016/S0960-8524(03)00148-2

Carvalho, A.F.A., Neto, P. de O., da Silva, D.F., Pastore, G.M., 2013. Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical

and enzymatic hydrolysis. Food Res. Int. 51, 75–85. https://doi.org/10.1016/j.foodres.2012.11.021

Cian, R.E., Garzón, A.G., Martínez-augustin, O., Botto, C.C., Drago, S.R., 2018. Antithrombotic Activity of Brewers' Spent Grain Peptides and their Effects on Blood Coagulation Pathways. J. Supercrit. Fluids 140, 85–90.

Clauser, N.M., Gutiérrez, S., Area, M.C., Felissia, F.E., Vallejos, M.E., 2015. Small-sized biorefineries as strategy to add value to sugarcane bagasse. Chem. Eng. Res. Des. 107, 137–146. https://doi.org/10.1016/j.cherd.2015.10.050

Connolly, A., O'Keeffe, M.B., Nongonierma, A.B., Piggott, C.O., FitzGerald, R.J., 2017. Isolation of peptides from a novel brewers spent grain protein isolate with potential to modulate glycaemic response. Int. J. Food Sci. Technol. 52, 146–153. https://doi.org/10.1111/ijfs.13260

Da Silva, S.S., Chandel, A.K., 2012. D-Xylitol Fermentative Production, Application and Commercialization.

Dávila, J.A., Rosenberg, M., Cardona, C.A., 2016. A biorefinery approach for the production of xylitol, ethanol and polyhydroxybutyrate from brewer's spent grain. AIMS Agric. Food 1, 52–66. https://doi.org/10.3934/agrfood.2016.1.52

De Faveri, D., Lambri, M., Converti, A., Perego, P., Del Borghi, M., 2002. Xylitol recovery by crystallization from synthetic solutions and fermented hemicellulose hydrolyzates. Chem. Eng. J. 90, 291–298. https://doi.org/10.1016/S1385-8947(02)00134-1

Duarte, L.C., Carvalheiro, F., Lopes, S., Marques, S., Pajaró, J.C., Gírio, F.., 2004. Comparison of Two Posthydrolysis Processes of Brewery's Spent Grain Autohydrolysis Liquor to Produce a Pentose-Containing Culture Medium †. Appl. Biochem. Biotechnol. 113–116, 1041–1058.

Ercili, D., Silventoinen, P., Sozer, N., Nordlund, E., 2018. Innovation: Plant Proteins as Food Ingredients. World Food Ingredients.

Finley, J.W., Walker, C.E., Hautala, E., 1976. Utilization of Press Water from Brewers Spent Grains. J. Sci. Food Agric. 27, 655–660.

Franceschin, G., Sudiro, M., Ingram, T., Smirnova, I., Brunner, G., Bertucco, A., 2011. Conversion of rye straw into fuel and xylitol: A technical and economical assessment based on experimental data. Chem. Eng. Res. Des. 89, 631–640. https://doi.org/10.1016/j.cherd.2010.11.001

Gerbrandt, K.L., 2014. The Impacts of Xylitol Production from Hemicellulose Residues: Process Design, Life Cycle, and Techno-Economic Assessment by. University of Toronto.

GMI, 2017. Prebiotics Market worth over $8.5 Bn by 2024.

Gómez, B., Gullón, B., Yáñez, R., Parajó, J.C., Alonso, J.L., 2013. Pectic oligosacharides from lemon peel wastes: Production, purification, and chemical characterization. J. Agric. Food Chem. 61, 10043–10053. https://doi.org/10.1021/jf402559p

Gómez, B., Miguez, B., Veiga, A., Parajó, J.C., Alonso, J.L., 2015. Production, Purification, and in Vitro Evaluation of the Prebiotic Potential of Arabinoxylooligosaccharides from Brewer's Spent Grain. J. Agric. Food Chem. 63, 8429–8438. https://doi.org/10.1021/acs.jafc.5b03132

González-García, S., Morales, P.C., Gullón, B., 2018. Estimating the environmental impacts of a brewery waste–based biorefinery: Bio-ethanol and xylooligosaccharides joint production case study. Ind. Crops Prod. 123, 331–340. https://doi.org/10.1016/j.indcrop.2018.07.003

Gorgens, J., Mandeagari, M., Farzad, S., Dafal, A., Haigh, K., 2016. Biorefinery approach to improve the suistanability of the South African sugar industry: Green Economy Research Report.

Gray, M.C., Converse, A.O., Wyman, C.E., 2007. Solubilities of Oligomer Mixtures Produced by the Hydrolysis of Xylans and Corn Stover in Water at 180 ° C. Ind. Eng. Chem. Res. 46, 2383–2391.

Gullón, P., González-Muñoz, M.J., Parajó, J.C., 2011. Manufacture and prebiotic potential of oligosaccharides derived from industrial solid wastes. Bioresour. Technol. 102, 6112–6119. https://doi.org/10.1016/j.biortech.2011.02.059

Hegy, S., Glagola, J., Tremblay, D., Mccarthy, D., 2013. Jump Start: Activated Economics in Aspen Plus ® V8.4.

Heikkila, H., Nurmi, J., Rahkila, L., Toyryla, M., 1992. Method for the production of xylitol. 5081026.

Hong, Y., Nizami, A.-S., Bafran, M.P., Saville, B.A., 2014. Impact of cellulase production on environmental and financial metrics for lignocellulosic ethanol. Biofuels, Bioprod. Biorefining 8, 743. https://doi.org/10.1002/BBB

Huige, N.J., 1994. Handbook of Brewing: In Food Science and Technology, in: Hardwick, W.A. (Ed.), Marcel Dekker Inc. CRC Press, pp. 501–550). https://doi.org/10.1002/9780470290118

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., Dudgeon, D., 2011. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol, National Renewable Energy Laboratory.

Ibbett, R., White, R., Tucker, G., Foster, T., 2019. Hydro-mechanical processing of brewer's spent grain as a novel route for separation of protein products with differentiated techno-functional properties. Innov. Food Sci. Emerg. Technol. 56, 102184. https://doi.org/10.1016/j.ifset.2019.102184

Ikram, S., Huang, L., Zhang, H., Wang, J., Yin, M., 2017. Composition and Nutrient Value Proposition of Brewers Spent Grain. J. Food Sci. 00, 1–11. https://doi.org/10.1111/1750-3841.13794

Ishiwaki, B.N., Murayama, H., Awayama, H., Kanauchi, O., Sato, T., 2000. Development of High Value Uses of Spent Grain by Fractionation Technology Q uarterly. MBAA Tech. Q. 37, 261–265.

Kemppainen, K., Rommi, K., Holopainen, U., Kruus, K., 2016. Steam explosion of Brewer's spent grain improves enzymatic digestibility of carbohydrates and affects solubility and stability of proteins. Appl. Biochem. Biotechnol. 1–15. https://doi.org/10.1007/s12010-016-2085-9

Kerby, C., Vriesekoop, F., 2017. An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3, 1–12. https://doi.org/10.3390/beverages3020024

Kolfschoten, R.C., Bruins, M.E., Sanders, J.P.M., 2014. Opportunities for small-scale biorefinery for production of sugar and ethanol in the Netherlands. Biofuels, Bioprod. Bioref. 1–12. https://doi.org/10.1002/bbb.1487

Larsen, J., Øtergaard Petersen, M., Thirup, L., Li, H.W., Iversen, F.K., 2008. The IBUS process - Lignocellulosic bioethanol close to a commercial reality. Chem. Eng. Technol. 31, 765–772. https://doi.org/10.1002/ceat.200800048

Lischeske, J.J., Crawford, N.C., Kuhn, E., Nagle, N.J., Schell, D.J., Tucker, M.P., McMillan, J.D., Wolfrum, E.J., 2016. Assessing pretreatment reactor scaling through empirical analysis. Biotechnol. Biofuels 9, 213. https://doi.org/10.1186/s13068-016-0620-0

Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. J. Inst. Brew. 4, 553–568. https://doi.org/10.1002/jib.363

Margulis, C., 2006. The Hazards of Genetically Engineered Foods. Environ. Health Perspect. 114, 153–155. https://doi.org/10.1289/ehp.8417

Martínez, E.A., Canettieri, E. V, Bispo, J.A.C., Giulietti, M., De, J.B., Silva, A., Converti, A., 2015. Strategies for xylitol purification and crystallization: A Review. Sep. Sci. Technol. 50, 2087–2098. https://doi.org/10.1080/01496395.2015.1009115

Misailidis, N., Campbell, G.M., C.Du, Sadhukhan, J., Mustafa, M., Mateos-Salvador, F., R.M.Weightman, 2009. Evaluating the feasibility of commercial arabinoxylan production in the context of a wheat biorefinery principally producing ethanol. Part 2. Process simulation and economic analysis. Chem. Eng. Res. Des. 87, 1239–1250. https://doi.org/10.1016/j.cherd.2008.12.027

Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment-A review. Biotechnol. Bioeng. 109, 1430–1442. https://doi.org/10.1002/bit.24464

Monin, T., 2016. Life Refreshment - Pilot for Environmentally Friendly, Efficient, Sustainable and Healthy products development: LIFE15 ENV/BE/000267.

Murthy, G.S., Sridhar, S., Sunder, M.S., Shankaraiah, B., Ramakrishna, M., 2005. Concentration of xylose reaction liquor by nanofiltration for the production of xylitol sugar alcohol. Sep. Purif. Technol. 44, 221–228. https://doi.org/10.1016/j.seppur.2005.01.009

Mussatto, S.I., 2014. Brewer's spent grain: A valuable feedstock for industrial applications. J. Sci. Food Agric. 94, 1264–1275. https://doi.org/10.1002/jsfa.6486

Mussatto, S.I., Moncada, J., Roberto, I.C., Cardona, C.A., 2013. Techno-economic analysis for brewer's spent grains use on a biorefinery concept: The Brazilian case. Bioresour. Technol. 148, 302–310. https://doi.org/10.1016/j.biortech.2013.08.046

Mussatto, S.I., Roberto, I.C., 2008. Establishment of the optimum initial xylose concentration and nutritional supplementation of brewer's spent grain hydrolysate for xylitol production by Candida guilliermondii. Process Biochem. 43, 540–546. https://doi.org/10.1016/j.procbio.2008.01.013

Mussatto, S.I., Roberto, I.C., 2005. Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J. Sci. Food Agric. 85, 2453–2460. https://doi.org/10.1002/jsfa.2276

Nieder-Heitmann, M., Haigh, K.F., Görgens, J.F., 2018. Process design and economic analysis of a biorefinery co-producing itaconic acid and electricity from sugarcane bagasse and trash lignocelluloses. Bioresour. Technol. 262, 159–168. https://doi.org/10.1016/j.biortech.2018.04.075

O'Donohue, M., 2014. Biocommodity Refinery Final Report FP7-241566.

Oliver, G., 2011. The Oxford Companion to Beer. Oxford University Press.

Özüdoğru, H.M.R., Nieder-Heitmann, M., Haigh, K.F., Görgens, J.F., 2019. Techno-economic analysis of product biorefineries utilizing sugarcane lignocelluloses: Xylitol, citric acid and glutamic acid scenarios annexed to sugar mills with electricity co-production. Ind. Crops Prod. 133, 259–268. https://doi.org/10.1016/j.indcrop.2019.03.015

Parajó, J.C., Dominguez, H., Domínguez, J.M., 1997. Improved xylitol production with Debaryomyces hansenii Y-7426 from raw or detoxified wood hydrolysates. Enzyme Microb. Technol. 21, 18–24. https://doi.org/10.1016/S0141-0229(96)00210-4

Petersen, A.M., Aneke, M.C., Görgens, J.F., 2014. Techno-economic comparison of ethanol and electricity coproduction schemes from sugarcane residues at existing sugar mills in Southern Africa. Biotechnol. Biofuels 7. https://doi.org/10.1186/1754-6834-7-105

Petersen, A.M., Franco, T., Görgens, J.F., 2018. Comparison of recovery of volatile fatty acids and mixed ketones as alternative downstream processes for acetogenisis fermentation. Biofuels, Bioprod. Biorefining 12.

Prista, C., Michán, C., Miranda, I.M., Ramos, J., 2016. The halotolerant Debaryomyces hansenii, the Cinderella of non-conventional yeasts. Yeast. https://doi.org/10.1002/yea.3177

Ramukhwatho, F., Seetal, A., Pienaar, H., 2016. NATSURV 1: Water and Wastewater Management in the Malt Brewing Industry (Ed 2) WRC Report No. TT 676/16. Pretoria.

Rivas, B., Torre, P., Dominguez, J.M., Converti, A., 2009. Maintenance and Growth Requirements in the Metabolism of Debaryomyces hansenii Performing Xylose-to-Xylitol Bioconversion in Corncob Hemicellulose Hydrolyzate. Biotechnol. Bioeng. 2009;102: 102, 1062–1073. https://doi.org/10.1002/bit.22155

Rivas, B., Torre, P., Domínguez, J.M., Perego, P., Converti, A., Parajó, J.C., 2003. Carbon material and bioenergetic balances of xylitol production from corncobs by Debaryomyces hansenii. Biotechnol. Prog. 19, 706–713. https://doi.org/10.1021/bp025794v

Robertson, J.A., I'Anson, K.J.A., Treimo, J., Faulds, C.B., Brocklehurst, T.F., Eijsink, V.G.H., Waldron, K.W., 2010. Profiling brewers' spent grain for composition and microbial ecology at the site of production. LWT - Food Sci. Technol. 43, 890–896. https://doi.org/10.1016/j.lwt.2010.01.019

Rosa, M., Beloborodko, A., 2015. A decision support method for development of industrial synergies : case studies of Latvian brewery and wood-processing industries. J. Clean. Prod. 105, 461–470. https://doi.org/10.1016/j.jclepro.2014.09.061

Sampaio, F.C., Passos, F.M.L., Passos, F.J.V., De Faveri, D., Perego, P., Converti, A., 2006. Xylitol crystallization from culture media fermented by yeasts. Chem. Eng. Process. Process Intensif. 45, 1041–1046. https://doi.org/10.1016/j.cep.2006.03.012

Schwencke, K. V, 2006. Sustainable, Cost-Effective, and Feasible Solutions for the Treatment of Brewers' Spent Grains. MBAA Tech. Q. 43, 199–202.

Skendi, A., Harasym, J., Galanakis, C.M., 2018. 7 – Recovery of high added-value compounds from brewing and distillate processing by-products, Sustainable Recovery and Reutilization of Cereal Processing. Elsevier Science. https://doi.org/10.1016/B978-0-08-102162-0.00007-1

Stiles, R.S., Herbert, H.S., 1977. Centrifugal Separation, Isolation and Characteristics of Brewery Spent Grains and Press Liquor. MBAA Tech. Q. 14, 21–34.

The Economist, 2017. Around the world, beer consumption is falling - Daily chart [WWW Document]. Econ. URL https://www.economist.com/graphic-detail/2017/06/13/around-the-world-beer-consumption-is-falling (accessed 9.10.18).

Towler, G.P., Sinnott, R.K., 2008. Chemical Engineering Design: Principles, Practice and Economics of Plant and Process Design. Elsevier.

Tucker, M.P., Nagle, N.J., Jennings, E.W., Ibsen, K.N., Aden, A., Nguyen, Q.A., Kim, K.H., Noll, S.L., 2004. Conversion of distiller's grain into fuel alcohol and a higher-value animal feed by dilute-acid pretreatment. Appl Biochem Biotechnol 113–116, 1139–1159. https://doi.org/10.1385/ABAB:115:1-3:1139

Ur-Rehman, S., Mushtaq, Z., Zahoor, T., Jamil, A., Anjum Murtaza, M., 2013. Xylitol; A review on Bio-production, Application, Health Benefits and Related Safety Issues. Crit. Rev. Food Sci. Nutr. 55, 37–41. https://doi.org/10.1080/10408398.2012.702288

Vallejos, M.., Chade, M., Mereles, E.B., Bengoechea, D.I., Brizuela, J.G., Felissia, F.E., Area, M.A., 2015. Xylitol bioproduction from sugarcane bagasse: Detoxification and fermenttion strategies. 3rd Iberoam. Congr. Biorefineries (CIAB), 2015, Concepción, Chile. https://doi.org/10.1017/CBO9781107415324.004

Vazquez, M.J., Alonso, J.L., Dominquez, H., Pajaro, J.C., 2000. Xylooligo-saccharides: manufacture and applications. Trends Food Sci. Technol. 11, 387–393.

Wan, N.-H.L., Xie, Y., Nancy Ho, 2003. Novel Simulated Moving Bed Technologies for Isolation of Sugars from Biomass Hydrolyzate.

Wang, B., Luo, Y., Myung, K.H., Liu, J.X., 2014. Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grains. Asian-Australasian J. Anim. Sci. 27, 832–840. https://doi.org/10.5713/ajas.2013.13668

Weger, A., Binder, S., Franke, M., Hornung, A., 2014. Solid Biofuel Production by Mechanical Pre-Treatment of Brewers' Spent Grain. Chem. Eng. Trans. 37, 661–666. https://doi.org/10.3303/CET1437111

Weger, A., Jung, R., Stenzel, F., Hornung, A., 2017. Optimized Energetic Usage of Brewers' Spent Grains. Chem. Eng. Technol. 40, 306–312. https://doi.org/10.1002/ceat.201600186

Wei, J., Yuan, Q., Wang, T., Wang, L., 2010. Purification and crystallization of xylitol from fermentation broth of corncob hydrolysates. Front. Chem. Eng. China 4, 57–64. https://doi.org/10.1007/s11705-009-0295-1

Wilkinson, S., Smart, K.A., James, S., Cook, D.J., 2016. Maximising high solid loading enzymatic saccharification yield from acid-catalysed hydrothermally-pretreated brewers spent grain. Biofuel Res. J. 3, 417–429. https://doi.org/10.18331/BRJ2016.3.2.7

Wooley, R.J., 1997. Continuous Countercurrent Chromatographic Separator for the Purification of Sugars from Biomass Hydrolysate - NREL/TP-580-23907.

Wooley, R.J., Putsche, V., 1996. Development of an ASPEN PLUS Physical Property Database for Biofuels Components. Victoria 1–38.

Zhang, H., Xiang-Xiang, Q., Aihua, C., Yuheng, Z., 2012. Method of producing xylitol and arabinose at the same time from hemicellulose hydrolysates. US 2012/0021467 A1. https://doi.org/US 20100322867A1

Züricher, C., Gruss, R., 1990. Recovering roughage from draft and product produced according to the method US4938986. Patent. 4938986.

Appendix: Chapter 6 – Techno-economic analysis ASPEN data

6.1 Upstream BSG drying

Processes before HTT are the same for all three scenarios.

6.1.1 Area A100 – Dewatering

Raw BSG is received with 85% moisture content and mechanically dewatered with a screw press creating two streams, a dried BSG (BPG) and a liquid press fraction containing soluble and suspended insoluble material. The pressed dried BSG is sent to HTT in area A200 while the liquid fraction sent for upgrading in area B100.

Figure 6A-1 Area A100 BSG dewatering

Screw press fractional split obtained by the screw press shows a large fraction of the starch and proteins are removed in the press liquid fraction.

Table 6A-1 Fractional split of components in the pressed dried BSG

Component ID	Basis	Value
XYLAN	Split fraction	0.93
LIGNIN	Split fraction	0.8
ASH	Split fraction	0.65
ACETATE	Split fraction	0.48
ARABINAN	Split fraction	0.8
CELLULOS	Split fraction	0.77
STARCH	Split fraction	0.54
PROTEIN	Split fraction	0.67
LIPIDS	Split fraction	0.8

6.1.2 Area B100 – Press liquid upgrade

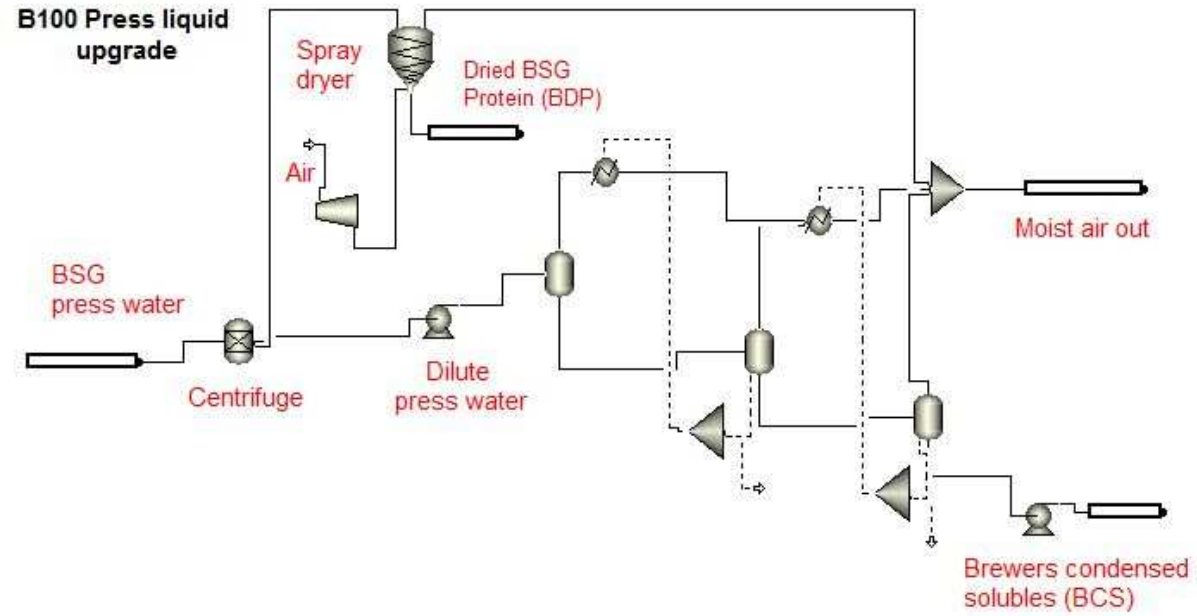

Figure 6A-2 Area B100 BSG press liquid upgrade

Component split obtained in the centrifuge solid liquid separation

Table 6A-2 Resulting solid insoluble component from split in centrifuge

Component ID	Basis	Value
XYLAN	Split fraction	0.24
LIGNIN	Split fraction	0.08
ASH	Split fraction	0.64
ACETATE	Split fraction	0.07
ARABINAN	Split fraction	0.08
CELLULOS	Split fraction	0.11
STARCH	Split fraction	0.55
PROTEIN	Split fraction	0.17

6.2 Scenario A – Xylitol production

6.2.1 Area A200 – Hydrothermal treatment

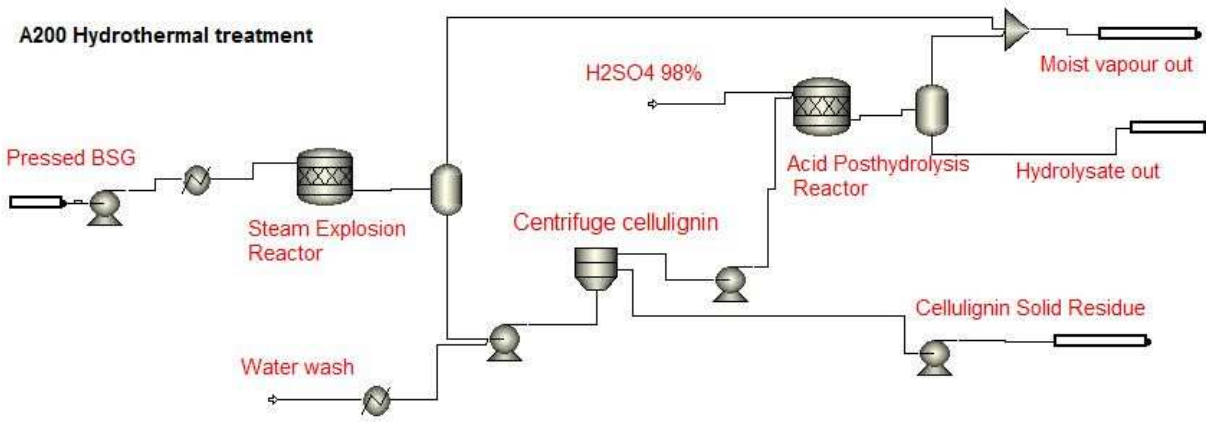

Figure 6A-3 Area A200 Hydrothermal treatment

HTT reactions and fractional conversions achieved.

Table 6A-3 Hydrothermal treatment reactions and yield

Reaction extent (kmol/h)	Reaction Stoichiometry	Product yield
0.038	CELLULOS (CISOLID) --> 2 H2O + HMF	0.010
0.369	ACETATE (CISOLID) --> ACACID	0.050
4.310	XYLAN (CISOLID) --> XYLOLIGO	0.742
0.354	H2O + XYLAN (CISOLID) --> XYLOSE	0.061
0.776	H2O + ARABINAN (CISOLID) --> ARABINOS	0.338
0.032	H2O + STARCH (CISOLID) --> GLUCOSE	0.037
3.160	PROTEIN (CISOLID) --> PROTSOL	0.450
2.542	LIGNIN (CISOLID) --> LIGNSOL	0.425
0.116	XYLAN (CISOLID) --> 2 H2O + FURFURAL	0.020
0.873	ARABINAN (CISOLID) --> ARABOLIG	0.380
0.778	ASH (CISOLID) --> CACL2	0.480
0.821	H2O + STARCH (CISOLID) --> GLUCOLIG	0.963

Acid posthydrolysis conducted at 125 ºC for 165 min using 0.5% H_2SO_4 (CSF 1.10)

Table 6A-4 Acid posthydrolysis of the hydrolysate reactions and yields

Reaction extent (kmol/h)	Reaction Stoichiometry	Product yield
0.0202	XYLAN(CISOLID) --> XYLOLIGO	0.98
0.8249	ARABOLIG + H2O --> ARABINOS	0.98
0.0127	ARABINAN(CISOLID) --> ARABOLIG	0.98
0.0000	STARCH(CISOLID) --> STARCH	0.98
0.7644	GLUCOLIG --> GLUCOSE	0.98
4.0733	XYLOLIGO + H2O --> XYLOSE	0.99
0.0305	ASH(CISOLID) + H2SO4 --> CASO4 + H2O	0.95

6.2.2 Area A300 - Detoxification

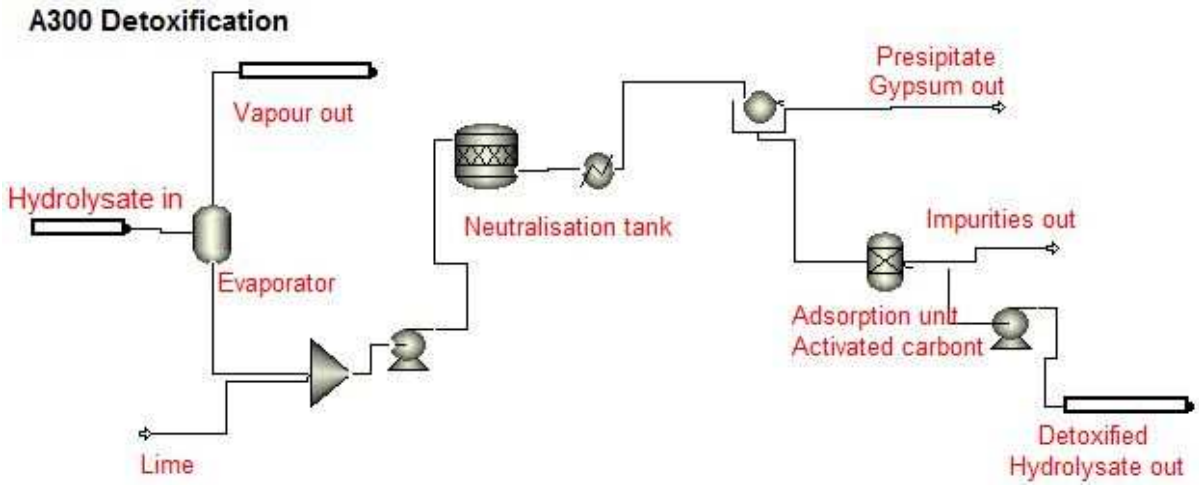

Figure 6A-4 Area A300 Detoxification

Hydrolysate neutralisation reactions

Table 6A-5 Neutralisation reactions

Reaction extent (kmol/h)	Reaction Stoichiometry	Product yield
0.96701	H2SO4 + CA(OH)2 --> 2 H2O + GYPSUM	1
0.03017	CASO4 --> GYPSUM	0.99

Table 6A-6 Resulting hydrolysate component yields after activated carbon treatment

Component ID	Basis	Value
ASH	Split fraction	0.95
H2O	Split fraction	1
GLUCOSE	Split fraction	0.95
XYLOSE	Split fraction	0.95
ETHANOL	Split fraction	0.95
CO2	Split fraction	0.95
LIGNSOL	Split fraction	0.27
ARABINOS	Split fraction	0.95
XYLOLIGO	Split fraction	0.95
ARABOLIG	Split fraction	0.95
N2	Split fraction	0.95
O2	Split fraction	0.95
ACACID	Split fraction	0.4
FURFURAL	Split fraction	0.78
H2SO4	Split fraction	0.95
CA(OH)2	Split fraction	0.95
FORMACID	Split fraction	0.8
HMF	Split fraction	0.36
LIPIDS	Split fraction	0.95
PROTEIN	Split fraction	0.95
PROTSOL	Split fraction	0.8
CASO4	Split fraction	0.95
GLUCOLIG	Split fraction	0.95
CACL2	Split fraction	0.95

6.2.3 Area A400 - Fermentation

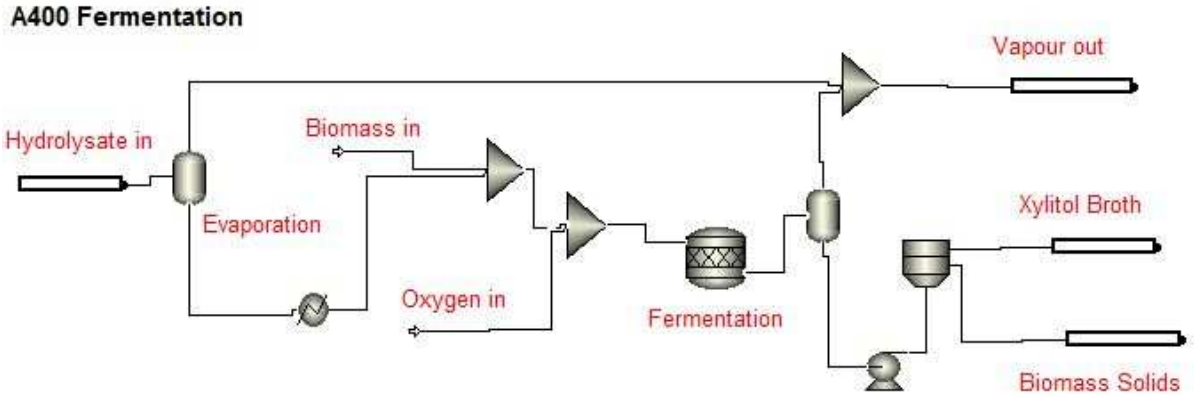

Figure 6A-5 Area A400 Fermentation

Fermentation of xylose and glucose with metabolic pathway reactions to produce biomass and xylitol (Rivas *et al.*, 2003, 2009).

Table 6A-7 Fermentation reactions

Reaction extent (kmol/hr)	Reaction Stoichiometry	Yield
0.00229	60 ARABINOS + 3 O2 + 24 H2O --> 54 ARABITOL + 30 CO2	0.10
0.05485	6 XYLOSE + 30 O2 --> 30 CO2 + 30 H2O	0.085
0.05227	60 XYLOSE + 3 O2 + 24 H2O --> 54 XYLITOL + 30 CO2	0.81
0.30055	1.095 XYLOSE + 0.2375 O2 + 0.13243 PROTSOL --> 5 BIOMASS + 0.475 CO2 + 2.5 H2O	0.85
0.01274	1.095 GLUCOSE + 0.158916 PROTSOL + 0.285 O2 --> 6 BIOMASS + 0.57 CO2 + 3 H2O	0.20
0.67651	GLUCOSE --> 2 ETHANOL + 2 CO2	0.97
0.01253	1.095 ARABINOS + 0.2375 O2 + 0.13243 PROTSOL --> 5 BIOMASS + 0.475 CO2 + 2.5 H2O	0.01
0.00891	ACACID + 2 O2 --> 2 CO2 + 2 H2O	0.35

6.2.4 Area A500 - Purification

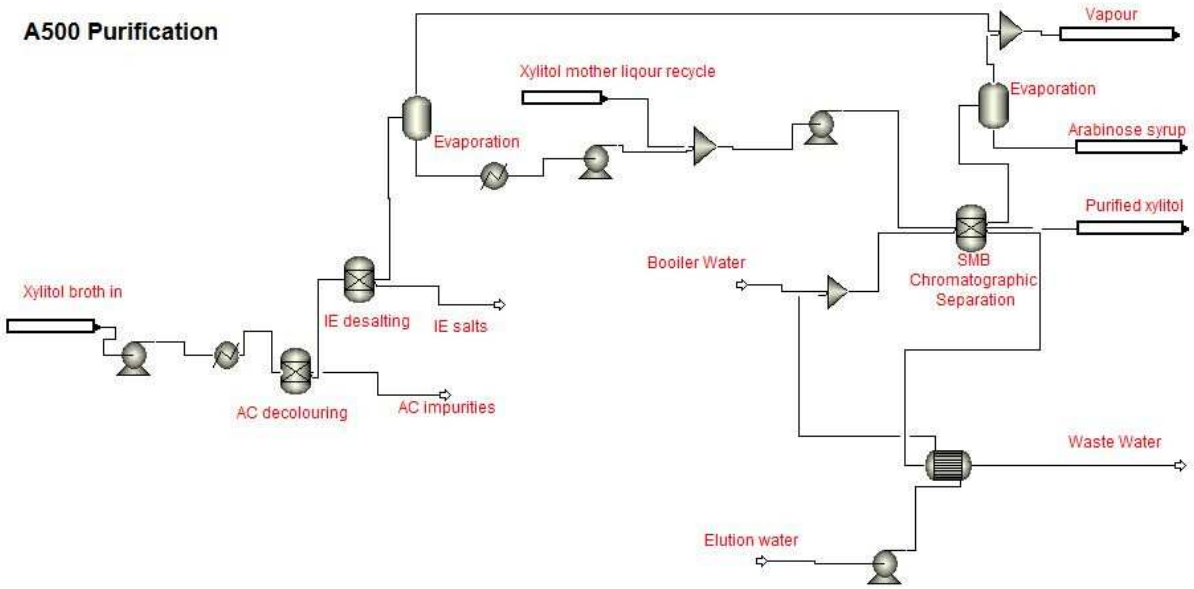

Figure 6A-6 Area A500 Purification

Firstly the broth purification is adsorption by activated carbon

Table 6A-8 Resulting hydrolysate component yields after activated carbon treatment

Component ID	Basis	Value
H2O	Split fraction	0.999
GLUCOSE	Split fraction	0.9
XYLOSE	Split fraction	0.9
ETHANOL	Split fraction	0.95
CO2	Split fraction	0.95
ACETATE	Split fraction	0.5
LIGNSOL	Split fraction	0.3
ARABINOS	Split fraction	0.9
XYLOLIGO	Split fraction	0.5
ARABOLIG	Split fraction	0.5
XYLITOL	Split fraction	0.95
N2	Split fraction	0.95
O2	Split fraction	0.95
ACACID	Split fraction	0.5
FURFURAL	Split fraction	0.95
H2SO4	Split fraction	0.9
CA(OH)2	Split fraction	0.95
FORMACID	Split fraction	0.8
HMF	Split fraction	0.35
LIPIDS	Split fraction	0.5
PROTSOL	Split fraction	0.5
CASO4	Split fraction	0.95
GLUCOLIG	Split fraction	0.5
CACL2	Split fraction	0.98
GYPSUM	Split fraction	0.98
ARABITOL	Split fraction	0.95

Secondly the absorption of the broth on ion exchange resin

Table 6A-9 Xylitol broth desalting with ion exchange resin

Component ID	Basis	Value
ASH	Split fraction	0.95
H2O	Split fraction	0.0001
GLUCOSE	Split fraction	0.05
XYLOSE	Split fraction	0.05
ETHANOL	Split fraction	0.05
CO2	Split fraction	0.05
LIGNSOL	Split fraction	0.5
ARABINOS	Split fraction	0.05
XYLOLIGO	Split fraction	0.05
ARABOLIG	Split fraction	0.05
XYLITOL	Split fraction	0.05
ACACID	Split fraction	0.95
FURFURAL	Split fraction	0.05
H2SO4	Split fraction	0.95
CA(OH)2	Split fraction	0.95
HMF	Split fraction	0.05
STARCH	Split fraction	0.05
LIPIDS	Split fraction	0.05
CASO4	Split fraction	0.95
GLUCOLIG	Split fraction	0.05
CACL2	Split fraction	0.95
GYPSUM	Split fraction	0.95
ARABITOL	Split fraction	0.1

Table 6A-10 Chromatographic separation of xylitol and sugars

Component ID	Basis	Xylitol fraction	Arabinose fraction
		Value	Value
H2O	Split fraction	0.28	0.08
XYLOSE	Split fraction	0.1	0.11
ARABINOS	Split fraction	0.1	0.75
XYLITOL	Split fraction	0.9	0.08
ARABITOL	Split fraction	0.1	0.45

6.2.5 Area A600 - Crystallisation

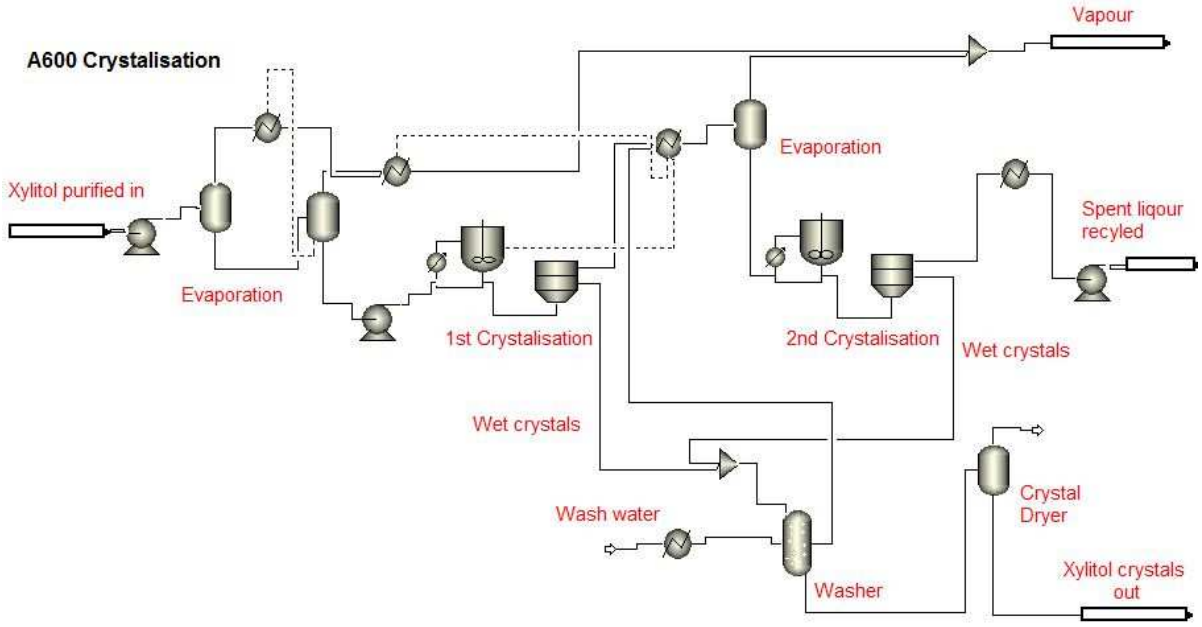

Figure 6A-7 Area A600 Crystallisation

6.3 Scenario B XOS production

6.3.1 Area A200 – Hydrothermal treatment

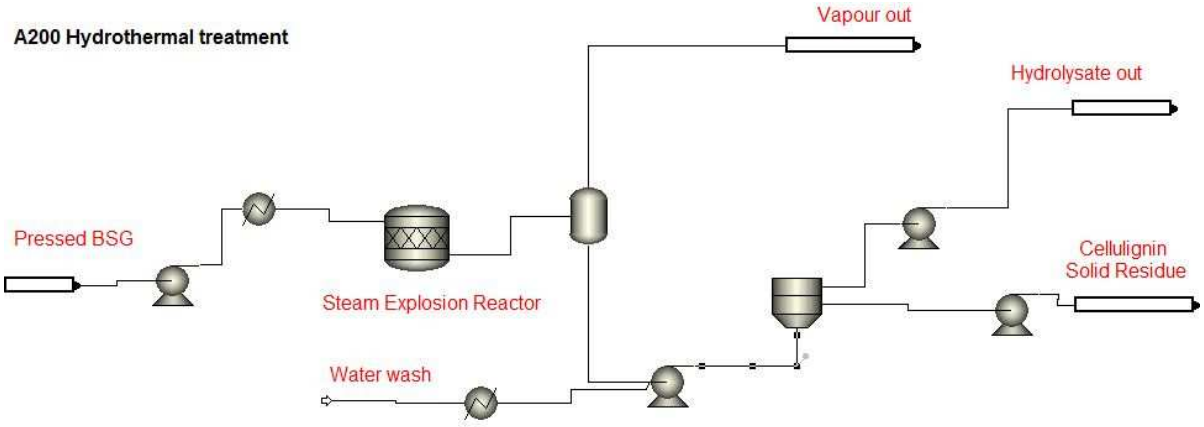

Figure 6A-8 A200 hydrothermal treatment

6.3.2 Area A350 – Diafiltration and enzymatic hydrolysis

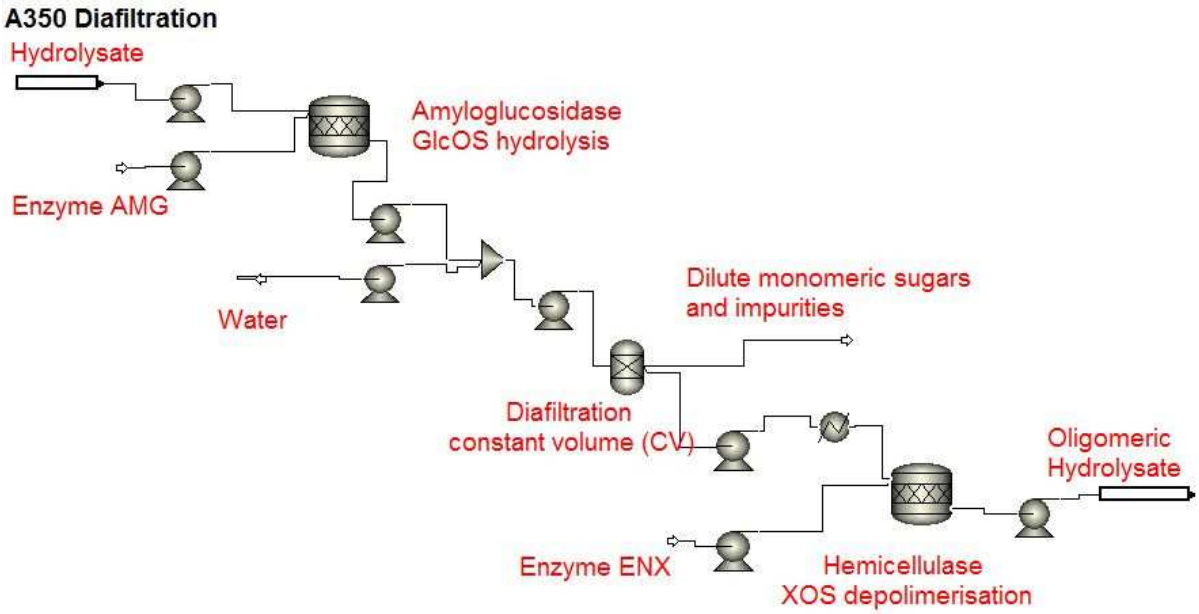

Figure 6A-9 Area 350 Diafiltration and enzymatic hydrolysis

Enzymatic hydrolysis – Amyloglucosidase

Table 6A-11 Enzymatic hydrolysis of GlcOS with amyloglucosidase

Reaction extent (kmol/hr)	Reaction Stoichiometry	Yield
0.77213	GLUCOLIG --> GLUCOSE	0.99

Diafiltration constant volume (CV) was conducted as described by Gómez et al., (2015)

Table 6A-12 Components recovery diafiltration constant volume

Component ID	Basis	Diafiltration Value
H2O	Split fraction	0.2
GLUCOSE	Split fraction	0.333
XYLOSE	Split fraction	0.286
CO2	Split fraction	0.2
LIGNSOL	Split fraction	0.709
ARABINOS	Split fraction	0.174
CELLOBIO	Split fraction	0.818
XYLOLIGO	Split fraction	0.834
ARABOLIG	Split fraction	0.756
N2	Split fraction	0.2
O2	Split fraction	0.2

FURFURAL	Split fraction	0.709
FORMACID	Split fraction	0.40
HMF	Split fraction	0.709
LIPIDS	Split fraction	0.709
PROTSOL	Split fraction	0.709
CASO4	Split fraction	0.709
GLUCOLIG	Split fraction	0.818
CACL2	Split fraction	0.25

Table 6A-13 Enzymatic hydrolysis of XOS with endoxylanase

Reaction extent (kmol/hr)	Reaction Stoichiometry	Yield
0.0041	GLUCOLIG --> GLUCOSE	0.64

6.3.3 Area A360 – Purification

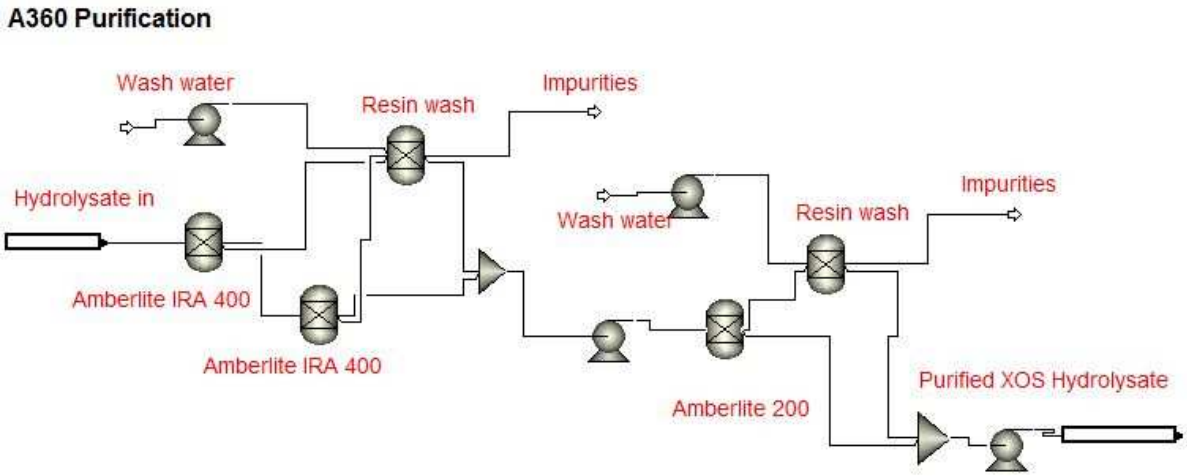

Figure 6A-10 Area A360 Purification of XOS

Table 6A-14 Ion exchange resin purification by IRA400 and 200 Amberlite

Component ID	Basis	IRA400 Value	Amber 200 Value
ASH	Split fraction	0.472527	0.304348
H2O	Split fraction	0.95	0.95
GLUCOSE	Split fraction	0.785714	0.909091
XYLOSE	Split fraction	1	1
ETHANOL	Split fraction	0	0
CO2	Split fraction	0.95	0.95
ACETATE	Split fraction	0.923077	0.769231
LIGNSOL	Split fraction	0.472527	0.304348
XYLOLIGO	Split fraction	0.928	0.883333

ARABOLIG	Split fraction	0.914286	0.878788
ACACID	Split fraction	0	0
FURFURAL	Split fraction	0	0
H2SO4	Split fraction	0.472527	0.304348
CA(OH)2	Split fraction	0.472527	0.304348
FORMACID	Split fraction	0	0
HMF	Split fraction	0	0
LIPIDS	Split fraction	0.472527	0.304348
PROTSOL	Split fraction	0.472527	0.304348
GLUCOLIG	Split fraction	0.923077	0.769231
CACL2	Split fraction	0.472527	0.304348
GYPSUM	Split fraction	0.472527	0.304348

6.3.4 Area A370 – XOS drying

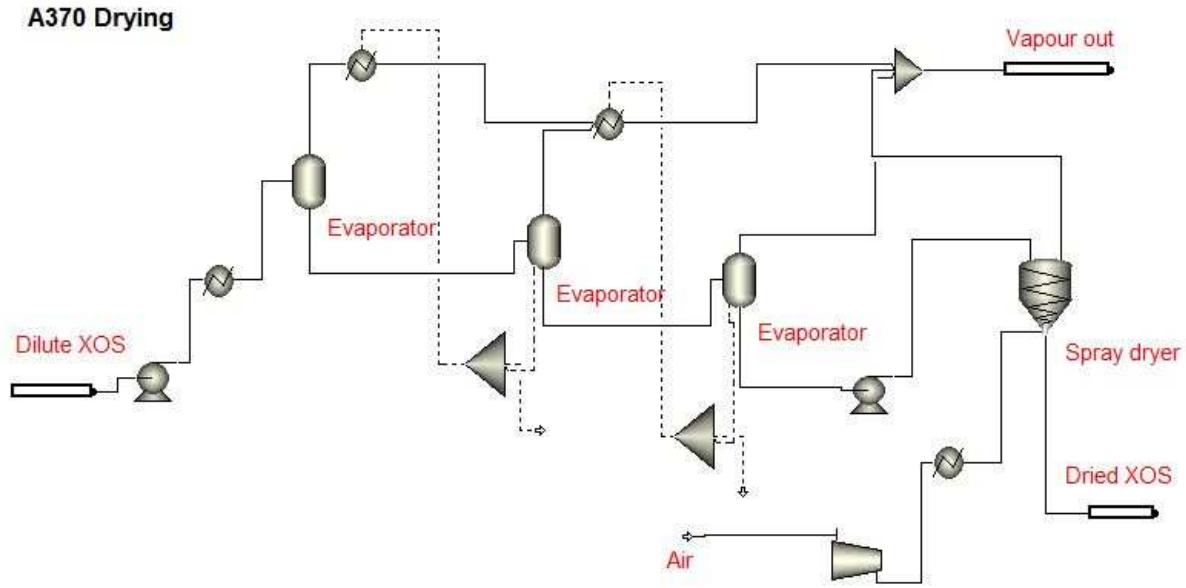

Figure 6A-11 Area A370 XOS drying

6.4 Scenario C Xylitol and XOS production

6.4.1 Area A200 – Hydrothermal treatment

HTT reactions and fractional conversions achieved.

Table 6A-15 Hydrothermal treatment reactions and yield

Reaction extent (kmol/h)	Reaction Stoichiometry	Product yield
0.023	CELLULOS (CISOLID) --> 2 H2O + HMF	0.006
0.369	ACETATE (CISOLID) --> ACACID	0.500
2.742	XYLAN (CISOLID) --> XYLOLIGO	0.472
2.172	H2O + XYLAN (CISOLID) --> XYLOSE	0.374
1.810	H2O + ARABINAN (CISOLID) --> ARABINOS	0.788
0.143	H2O + STARCH (CISOLID) --> GLUCOSE	0.167
4.810	PROTEIN (CISOLID) --> PROTSOL	0.685
1.884	LIGNIN (CISOLID) --> LIGNSOL	0.315
0.163	XYLAN (CISOLID) --> 2 H2O + FURFURAL	0.028
0.471	ARABINAN (CISOLID) --> ARABOLIG	0.205
0.802	ASH (CISOLID) --> CACL2	0.500
0.688	H2O + STARCH (CISOLID) --> GLUCOLIG	0.806

6.4.2 Area A300 – Detoxification

Area A300 is similar for scenario A and C, yet with less acid for neutralisation in scenario C

Table 6A-16 Neutralisation reactions

Reaction extent (kmol/h)	Reaction Stoichiometry	Product yield
0.3959097	H2SO4 + CA(OH)2 --> 2 H2O + GYPSUM	1
0	CASO4 --> GYPSUM	0.99

6.4.3 Area A400 – Fermentation

Fermentation follows similar reaction paths compared to scenario A.

Table 6A-17 Fermentation reacti0ons

Reaction extent (kmol/hr)	Reaction Stoichiometry	Yield
0.002415	60 ARABINOS + 3 O2 + 24 H2O --> 54 ARABITOL + 30 CO2	0.1
0.023333	6 XYLOSE + 30 O2 --> 30 CO2 + 30 H2O	0.085
0.022235	60 XYLOSE + 3 O2 + 24 H2O --> 54 XYLITOL + 30 CO2	0.81
0.127852	1.095 XYLOSE + 0.2375 O2 + 0.13243 PROTSOL --> 5 BIOMASS + 0.475 CO2 + 2.5 H2C	0.85
0.011965	1.095 GLUCOSE + 0.158916 PROTSOL + 0.285 O2 --> 6 BIOMASS + 0.57 CO2 + 3 H2O	0.2
0.635436	GLUCOSE --> 2 ETHANOL + 2 CO2	0.97
0.013233	1.095 ARABINOS + 0.2375 O2 + 0.13243 PROTSOL --> 5 BIOMASS + 0.475 CO2 + 2.5 H2O	0.01
0.014818	ACACID + 2 O2 --> 2 CO2 + 2 H2O	0.35

6.4.4 Area 350 – Filtration

Diafiltration under volume reduction (VR) as described by (Gómez *et al.*, 2013) and nanofiltration concentration described by (Ajao *et al.*, 2015). Monomeric xylose is recovered from the filtrate from 1kDa molecular weight cut-off membrane which is combined with diafiltration CV filtration filtrates. The retentate hydrolysate is passed to diafiltration using constant volume for further purification. Filtrates from both stages are combined and passed through 500 Da nanofiltration membranes to recycle oligomers while monomeric sugars are passed to 100-200 Da nanofiltration membranes for concentration in a 75% volume reduction.

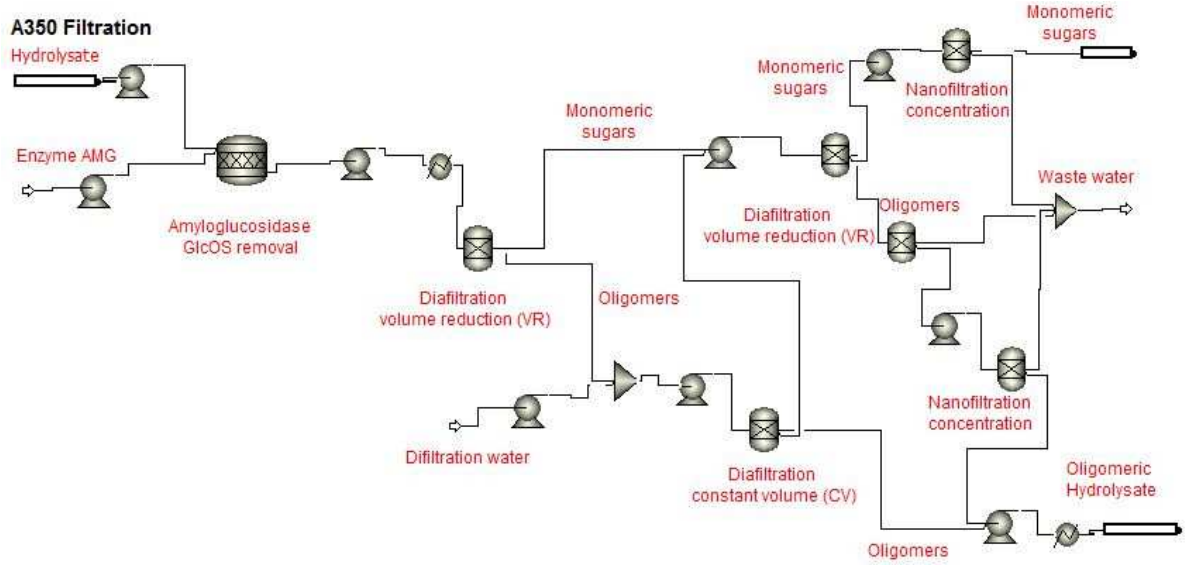

Figure 6A-12 Area 350 Filtration separating monomeric xylose sugars from oligomeric XOS

Table 6A-18 Enzymatic hydrolysis of GlcOS with amyloglucosidase

Reaction extent (kmol/hr)	Reaction Stoichiometry	Yield
0.63110	GLUCOLIG --> GLUCOSE	0.99

Table 6A-19 Diafiltration volume reduction components recovery in cleaned hydrolysate and concentration volume reduction with nanofiltration

Component ID	Basis	Diafiltration Volume reduction	Nanofiltration concentration
		Value	Value
H2O	Split fraction	0.72	0.25
GLUCOSE	Split fraction	0.11	0.99
XYLOSE	Split fraction	0.286	0.99
CO2	Split fraction	0.2	0.99
LIGNSOL	Split fraction	0.709	0.99

ARABINOS	Split fraction	0.174	0.99
CELLOBIO	Split fraction	0.818	0.99
XYLOLIGO	Split fraction	1	0.99
ARABOLIG	Split fraction	0.87	0.99
N2	Split fraction	0.2	0.99
O2	Split fraction	0.2	0.99
FURFURAL	Split fraction	0.33	0.99
H2SO4	Split fraction	0.25	0.99
CA(OH)2	Split fraction	0.33	0.99
FORMACID	Split fraction	0.33	0.99
HMF	Split fraction	0.33	0.99
LIPIDS	Split fraction	0.709	0.99
PROTSOL	Split fraction	0.75	0.99
CASO4	Split fraction	0.33	0.99
GLUCOLIG	Split fraction	0.818	0.99
CACL2	Split fraction	0.33	0.99

6.5 Summary of technical process product recoveries

Table 6A-20 Comparison between scenarios for production of xylitol and XOS: Process product recoveries, purity and stream TDS

	Scenario A - Xylitol						**Scenario B - XOS**				
		Product Xylan/Xylitol						**Product XOS+ArOS**			
Unit		**kg/h[b]**	**Yield**	**Purity**	**TDS%**	**Unit**		**kg/h[b]**	**Yield**	**Purity**	**TDS%**
	Feedstock	825.1						1204.4			
A100	Screw press dewat	767.4	93%					1070.8	89%		
A200	Hydrothermal trtm[a]	616.2	80%	22%	19%			650.4	61%	35%	8%
	Xylitol production						**XOS production**				
A200	Acid posthydrolysis	582.6	95%	33%	9%	A350	ENZ HD AML	650.4	100%	35%	9%
A300	EV/Neutr/AC	511.5	88%	42%	13%	A350	Diafl CV	533.9	82%	44%	6%
A400	Fermentation	478.9	81%	41%	17%	A350	ENZ HD Xyl	533.9	100%	44%	6%
A500	AC/IE/EV	415.4	87%	64%	41%	A360	IE Amb400 +200	527.0	99%	82%	3%
A500	Chromatography	373.9	90%	95%	4%	A370	XOS Drying	527.0	100%	82%	93%
A600	Crystallisation	342.3	92%	100%	100%						
	Overall recovery		**37% Xylan**					**52%Xylan/22% Arabinan**			

	Scenario C - Xylitol & XOS										
		Product Xylan/Xylitol						**Product XOS+ArOS**			
Unit		**kg/h[b]**	**Yield**	**Purity**	**TDS%**	**Unit**		**kg/h[b]**	**Yield**	**Purity**	**TDS%**
A100	Feedstock	825.1						1204.4			
A100	Screw press dewat	767.4						1070.8			
A200	Hydrothermal trtm[a]	616.7	85%	21%	19%			424.4	40%	13%	9%
A300	EV/Neutralisation	601.6	93%	30%	25%			393.3	93%	19%	25%
	Xylitol production						**XOS production**				
	Filtration VR CV	217.6	36%	31%	21%	A350	ENZ HD AML	393.3	100%	19%	25%
A400	Fermentation	181.8	73%	26%	22%	A350	Filtration VR	385.8	98%	32%	22%
A500	AC/IE/EV	177.6	98%	45%	43%	A350	Diafiltration CV	361.6	94%	38%	12%
A500	Chromatography	159.9	90%	89%	4%	A360	IE Amb400 +200	356.9	99%	80%	6%
A600	Crystallisation	145.5	91%	100%	100%	A370	XOS Drying	356.9	100%	80%	93%
	Overall recovery		**18% Xylan**					**37% Xylan/13% Arabinan**			

a Hydrolysate outlet assuming no dilution; b Dry basis
EV- Evaporation; AC – Activated carbon; IE – Ion exchange; Diafl – Diafiltration; VR – Volume reduction; CV – Constant volume
ENZ HD – Enzymatic hydrolysis; AML – Amyloglucosidase; EndX - Endoxylanase

6.6 Raw materials cost breakdown

6.6.1 Scenario A

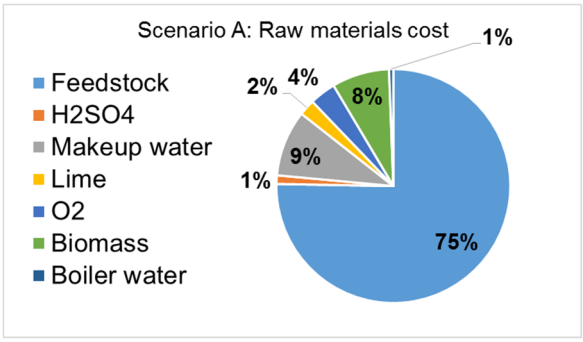

Figure 6A-13 Scenario A: Raw material cost breakdown

6.6.2 Scenario B

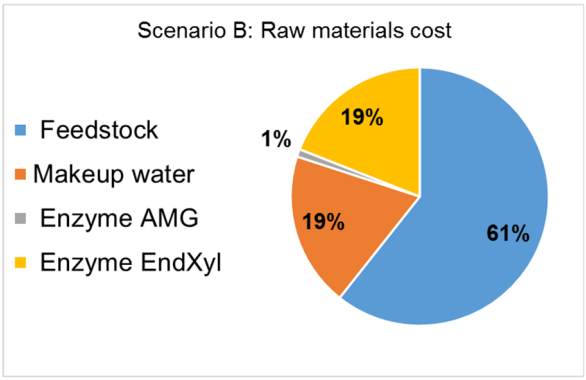

Figure 6A-14 Scenario B: Raw materials breakdown

6.6.3 Scenario C

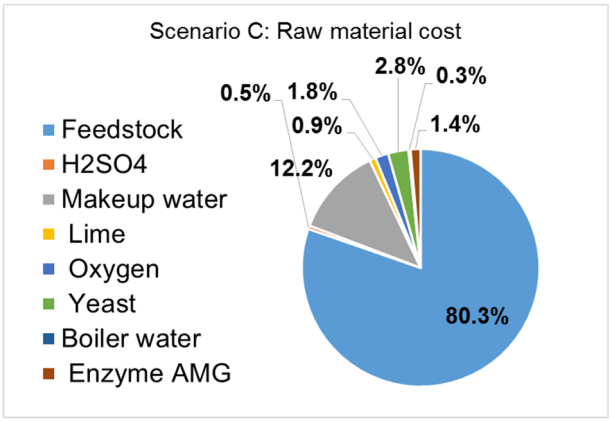

Figure 6A-15 Scenario C: Raw material cost breakdown

6.7 Process utilities break down for scenarios

6.7.1 Scenario A

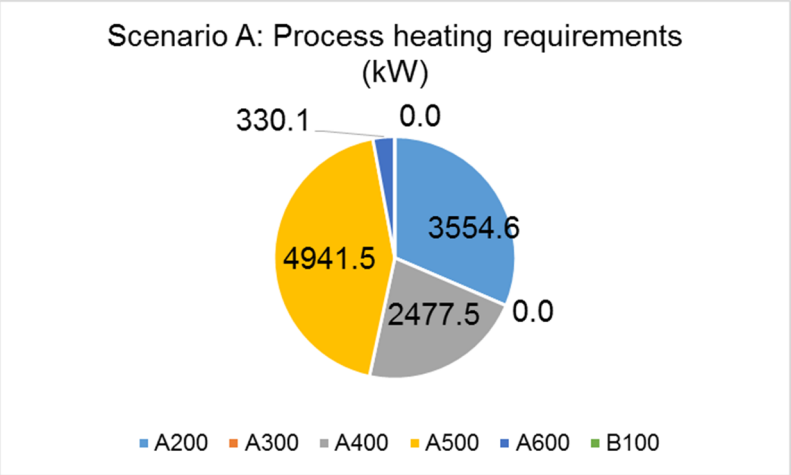

Figure 6A-16 Process heat requirements per process area

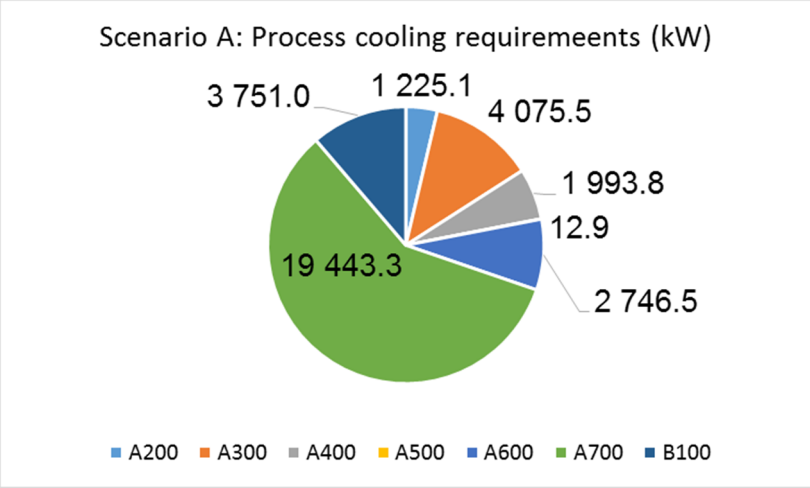

Figure 6A-17 Cooling requirements per process area

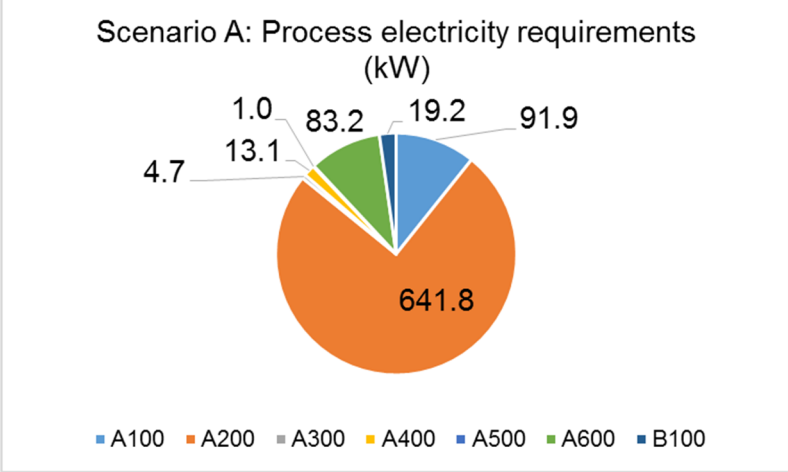

Figure 6A-18 Electricity requirements per process area

6.7.2 Scenario B

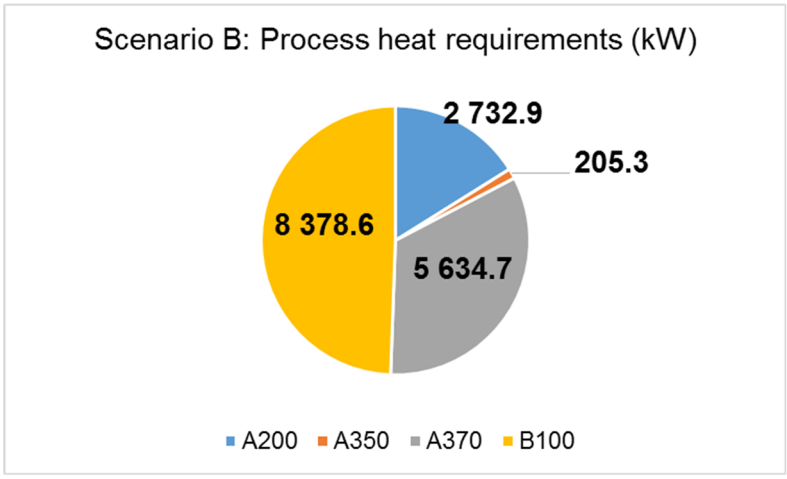

Figure 6A-19 Process heat requirements per process area

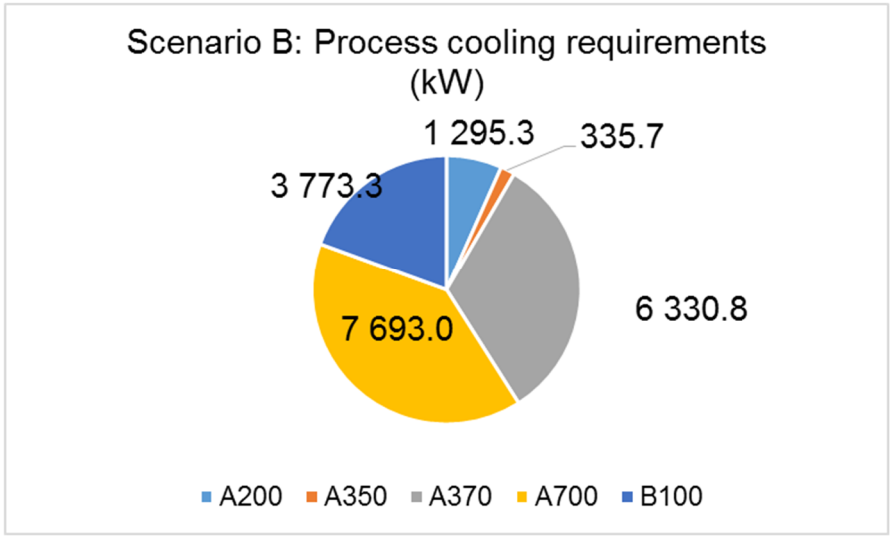

Figure 6A-20 Cooling requirements per process area

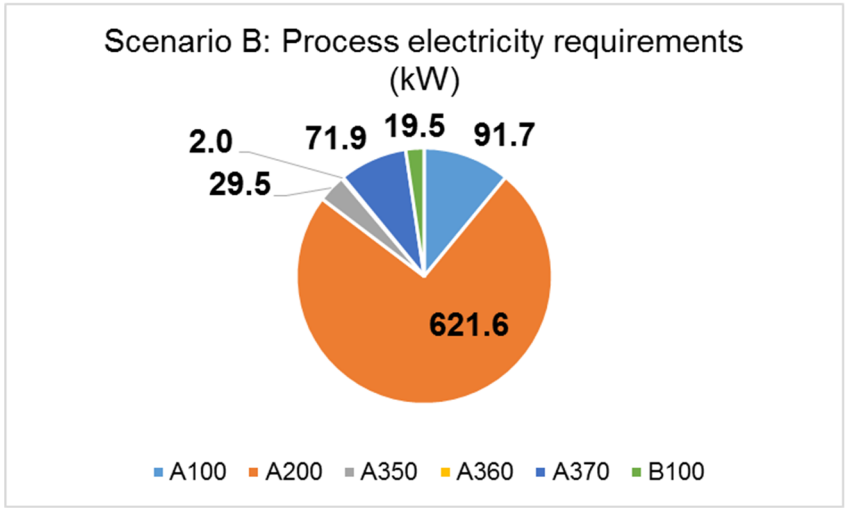

Figure 6A-21 Electricity requirements per process area

6.7.3 Scenario C

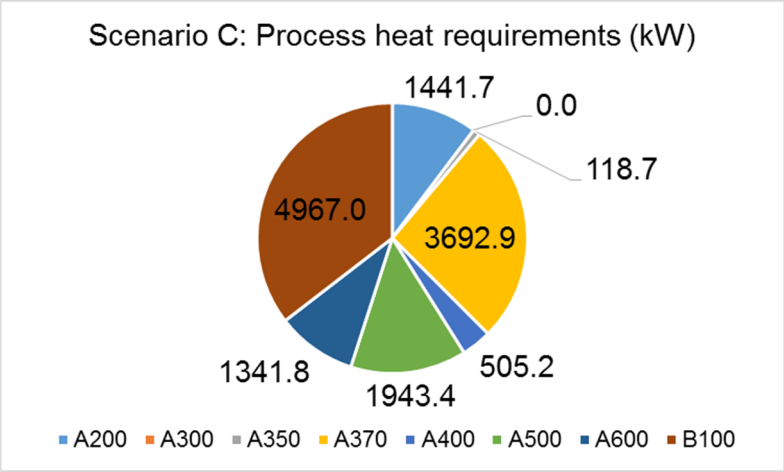

Figure 6A-22 Process heat requirements per process area

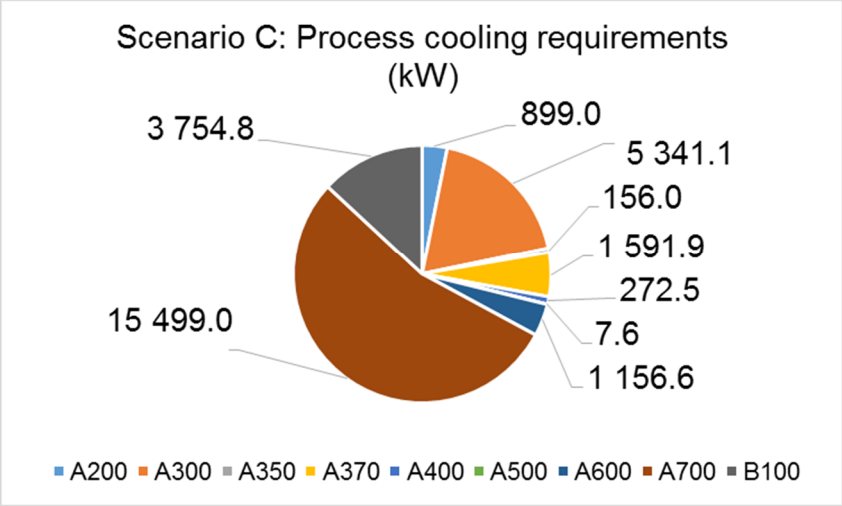

Figure 6A-23 Cooling requirements per process area

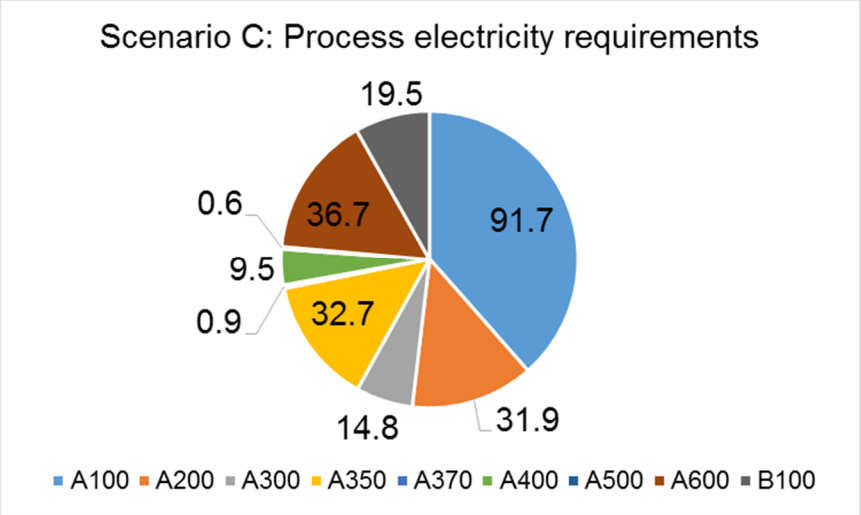

Figure 6A-24 Electricity requirements per process area

6.8 Summary of process equipment purchased and installed cost for the scenarios

6.8.1 Equipment list for scenario A

Table 6A-21 Equipment list for Scenario A: Xylitol production

Equipment Title	Source	Description	kW	Mate	Qty	$	Year of Quote	Scaling Variable	Scaling Val	Units	Scaling Exp	Inst Factor	New Val	Size Ratio	Scaled P cost	Purch Cost in PY	Inst Cost in PY
	NREL	BSG screw conw	**7.4**	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	21770	0.76	16064.22	18563.6	$31 558
A100.PLPOMP	ASPEN	Liquid press fraction pump				6100	2018									6100	53700
A100.SCPR	Alibaba	Screw press HDL-304	**83**	304SS	3	46000	2018	dry mass flow	6250	kg/h	0.8	1.5	6250	1	46000	138000	$207 000
							Area 100 Total installed cost	**$292 258**									
200.B1	ASPEN	Heat exch cellulignin			1	9800	2018									9800	60400
A200.B8	ASPEN	Centrifuge cellulignin	16.4		1	688800	2018									688800	1068700
A200.B2-flash vessel	ASPEN	Posthydrolysis reactor flash vessel		304SS	1	40200	2018									40200	180100
A200.B6	ASPEN	Hydrolysate pump out		304SS	1	5800	2018									5800	51400
A200.B9	ASPEN	Condenser reactor flash		304SS	1	13000	2018									13000	72400
A200.SREAC	ASPEN	Steam treatm reactor system	604.77	304SS	1	6604133	2018	mass flow	83333	Units	0.6	1.5	21770	0.261	2951486	2951486	4427229
	NREL	Cellulignin screw conveyor	**2.5**	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	4331	0	4414	5101	8672
A200.SREAC	NREL	Acid hydrolysis reactor 2 h 50m3		316SS	3	203000	2009	mass flow	264116	kg/h	0.7	2	24321	0.092	38232	132541	265082
	NREL	Tank agitators x3 75hp	**15.0**	SS	3	90000	2009	mass flow	264118	kg/h	0.5	1.5	24321	0.092	27311	94680	142020
	NREL	Sulfuric acid tank 24 hr resi		plastic	1	6210	2009	mass flow	1984	kg/h	0.7	0.4	114.00	0.057	841	972	389
	NREL	Sulfuric acid pump 8 GPM 245 FT		316SS	1	8000	2009	mass flow	3720	kg/h	0.8	2.3	100.00	0.027	443	512	1178
															Area 200 Total installed cost		**$6 277 568**
A300.B3	ASPEN	hydrolysate to detox pump			1	5600										5600	42900
A300.B4	ASPEN	heat loss out			1	9800										9800	58100
A300.B5	ASPEN	pump hydrolysate out			1	6700										6700	44100
		Activated carbon bed			1	0.9	2013		296	kg		1.1			266.4	283.211	311.5

A300 AC	ASPEN	AC bed			1	55200									55200	282600
	ASPEN	Evaporator			1	31200									31200	176400
	ASPEN	Evaporator			1	38400									38400	198000
	NREL	Lime screw convev			1	20000	2009	mass flow	28630	kg/h	0.8	1.7	89	0.003 1	197.28	227.97	1000
A300.DETOXTAN	NREL	1 hr 15m3		304SS	1	236000	2009	mass flow	410369	kg/h	0.7	2	11600	0.03	19445	22470	44941
Detox agitator	NREL	Agitator 10 Hp lotus	**0.75**	SS	1	21900	2009	mass flow	410369	kg/h	0.7	2	11600	0.03	1804	2085	4170
A300.GYPSFIL	ASPEN	Ryde DNY Rotary vacuum drum filter dewatering belt press	**2.6**	304SS	1	50000	2018	mass flow	450	kg/h	0.8	1.7	465	1	50000	50000	85000
															Area 300 Total installed cost		**$937 523**
A400.B1	ASPEN	Pump out section A500		304SS	1	4900	2018									4900	39700
A400.B2	ASPEN	Holding flash tank		304SS	1	27400	2018									27400	146900
A400.B4	ASPEN	Fermenter flash tank		304SS	1	33700	2018									33700	176000
A400.B5	ASPEN	Condenser evaporator		304SS	1	9700	2018									9700	59700
A400.B6	ASPEN	Fermenter Broth pump out		304SS	1	4900	2018									4900	39700
A400.EVAPXYL	ASPEN	Evaporator		304SS	1	42100	2018									42100	147100
A400.EXHFERM	ASPEN	Heatexch cooler fermenter		304SS	1	10800										10800	61000
A400.XYLCENT	ASPEN	Centrifuge biomass	6.1	304SS	1	459200										459200	712500
	NREL	Seed fermenter 100l		304SS	2	37700	2009		1		0.7	1.8	1	1	37700	87131	156836
	NREL	Seed fermenter agitator	**5.6**	SS	1	26000	2009	mass flow	1		0.5	1.5	1	1	26000	30045	45068
	NREL	Seed fermenter pump	**0.75**	304SS	1	8200	2009	mass flow	43149		0.8	2.3	7361	0	1992	2302	5296
A400.XYLFER	NREL	Fermenter 60h 100m3		304SS	6	844000	2018	volume	4000	m3	0.7	2	100	0.025	63811.7	382870.3	765741
																Area 400	**$2 355 540**
A500.A	ASPEN	Evaporator		304SS	1	27400	2018									27400	146900
A500.B1	ASPEN	Fresh water pump SMB		304SS	1	5700	2018									5700	47200
		Resin			1	4.7	2003		578	kg		1.1			2716.6	4075.58	4483.13
A500.B2	ASPEN	IE desalting column		304SS	1	289600	2018	flow rate	7430	kg/h	0.7	2.49	6691	0.90	269122.6	269122.6	670948
A500.B9	ASPEN	Feed pump SMB		304SS	1	5600	2018									5600	34000
A500.CHRSEP	NREL	SMB 9 zone			1	3120000	1997	feed flow rate l/min	260	l/min	0.8	1.5	26.35	0.1	499805	779902	1169854

	Wan	SMB 5 zone			1	1000000	2003	feed flow rate l/min	189	l/min	0.8	1.5	26.35	0.1	206755	
A500.EVAPCHR	ASPEN	Evaporator		304SS	1	33000	2018									33000	191300
A500.HXCHRSEP	ASPEN	Heat exch waste heat SMB			1	22500	2018									22500	89200
		Activated carbon bed			1	0.9	2013		231	kg		1.1			207.9	221.02	243.122
A500.MF	ASPEN	Activated carbon bed		304SS	1	289600	2018	flow rate	7430	kg/h	0.7	2.49	6961	0.94	276679.2	276679.2	689787
																Area 500	**$3 043 915**
A600.B1	ASPEN	Feed pump evaporator		304SS		5800										5800	43100
A600.B13	ASPEN	Centrifuge 1	0.83	304SS		128200	2018									128200	199400
A600.B17	ASPEN	Recycle pump		304SS		4900	2018									4900	31500
A600.B3	ASPEN	Centrifuge 2	0.31	304SS		128200	2018									128200	199400
A600.B5	ASPEN	Wash water cooler		304SS		0	2018		14	kg/h						0	0
A600.B7	ASPEN	Crystalliser feed pump		304SS		4900	2018									4900	31500
A600.CRDRIER	ASPEN	Crystal dryer		304SS		27700	2018									27700	162400
A600.CREVAP	ASPEN	Second stage evaporator		304SS		33700	2018									33700	168400
A600.DEXFL2	ASPEN	Heat exch vapor condenser		304SS		7700	2018									7700	44000
A600.FLEVB1	ASPEN	Evaporator		304SS		33600	2018									33600	160200
A600.FLEVB2	ASPEN	Evaporator		304SS		38400	2018									38400	188400
A600.WASHER	ASPEN	Drum washer		CS		93800	2018									93800	155100
A600.XYLCRT1	ASPEN	Crystalliser		304SS		101600	2018									101600	160200
A600.XYLCRT2	ASPEN	Crystalliser		304SS		58300	2018									58300	97900
																Area 600	**$1 641 500**
A700.B2	ASPEN	Flash condenser		304SS		69400	2018									69400	257300
						75000	2018									75000	182800
																Area 700	**$257 300**
B100.B1	ASPEN	Clear press water pump		304SS		5700	2018									5700	43000
B100.B9	ASPEN	BCS pump		304SS		4900	2018									4900	33300
B100.CNTRFP	ASPEN	Solid liquid separator	17.2	304SS		918400	2018	Mass flow	19698							918400	1425000
B100.DRYBSGPD	ASPEN	Spray drier		304SS		42000	2018									42000	210200

B100.EVAP1	ASPEN	Evaporator	304SS	38200	2018	38200	165800
B100.EVAP2	ASPEN	Evaporator	304SS	39700	2018	39700	190700
B100.EVP3	ASPEN	Evaporator	304SS	27400	2018	27400	139800
						Area B100	**$ 2 207 800**
							$17 013 404

6.8.2 Equipment list for scenario B

Table 6A-22 Equipment list for Scenario B: XOS production

Equipment Title	Source	Description	kW	Material	Qty	$	Year of Quote	Scal Var	Scaling Val	Units	Scale Exp	Inst Factor	New Val	Size Ratio	Scaled P cost	Purch Cost iPY	Inst Cost inPY
	NREL	BSG screw conw	8.5	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	21770	0.76	16064	18563.6	$31 558
A100.PLPOMP	ASPEN	Liquid press fraction pump				6100	2018									6100	53700
A100.SCPR	Alibaba	Screw press HDL-304	83	304SS	3	46000	2018	mass flow	6250	kg/h	0.8	1.5	6250	1	46000	138000	$207 000
							Area 100 Total installed cost										**$292 258**
A200.B1	ASPEN	Heat exch cellulignin			1	9800	2018									9800	60400
A200.B8	ASPEN	Centrifuge cellulignin	16.36		1	688800	2018									688800	1068700
A200.B6	ASPEN	Hydrolysate pump out		304SS	1	5800	2018									5800	51400
A200.B9	ASPEN	Condenser reactor flash		304SS	1	13000	2018									13000	72400
A200.SREAC	ASPEN	Steam treatm reactor system	604.8	304SS	1	6604133	2018	mass flow	83333	Units	0.6	1.5	21770	0.26	2951486	2951486	4427229
	NREL	Cellulignin screw conveyor	1.287	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	4331	0.151	4414.11	5100.88	8672
														Area 200 Total installed cost			**$5 688 800**
A350.B10	ASPEN	Pump dilution water diafiltration		304SS	1	10300										10300	84500
A350.B1	NREL	Enzyme EndXyl reactor		304SS	1	480000	2009	volume	1000	m3	0.7	2	1000	1.0	480000.0	554681	1109362
	NREL	Agitator	6	316SS	1	11000	2009		1		1	1.5	1	1	11000	12711	19067
A350.B4	ASPEN	Enzyme pump		304SS	1	4900	2018									4900	29000
A350.B13	ASPEN	Hydrolysate Enzy Preheater			1	8200										8200	54200
A350.B15	ASPEN	Pump diafiltration			1	10900										10900	91000
A350.B17	ASPEN	Heatexch cooler diafiltration			1	13000	2018									13000	69700
A350.B2	ASPEN	Feed pump enzymatic hydrolysis		304SS	1	6400										6400	54000
A350.B3	ASPEN	Pump outlet enzymatic hydrolysis		304SS	1	6300										6300	53900
	ASPEN	Heatexch oligos out			1	4900										4900	29000
A350.B7	ASPEN	Enzyme AML pump			1	4900	2018									4900	29000

A350.DIACV	ASPEN	Diafiltration membrane			1	3000	2005	m2	30	m2	1	1.5	1665	55.5	166472.9	214437.9	321657
A350.ENZRHAML	NREL	Enzyme AML reactor		304SS	1	480000	2009	volume	1000	m3	0.7	2	1000	1.0	480000.	554681	1109362
	NREL	Agitator	6	316SS	1	11000	2009		1		1	1.5	1	1	11000	12711	19067
																Area 350	**$3 072 815**
A360.B1	ASPEN	Pump washing 2		304SS	1	4900										4900	39700
A360.B2	ASPEN	Transfer pump		304SS	1	6100										6100	53600
A360.B3	ASPEN	Pump hydrolysate out		304SS	1	7500										7500	55500
A360.B4	ASPEN	Pump washing 1		304SS	1	4900										4900	39700
		Resin					2003		4.7								
A360.GAMBER1	ASPEN	Amberlite IRA 400 bed		304SS	1	756200	2018					2.0202				756200	1527700
A360.GAMBER1B	ASPEN	Amberlite IRA 400 bed		304SS	1	756200	2018									756200	1527700
A360.GAMBER2	ASPEN	Amberlite 200		304SS	1	756200	2018	flowrate	21066	kg/h		2.02	19854	0.942		712693	1439806
																Area 360	$ 4 683 706
A370.B6	ASPEN	Evaporator feed pump		304SS	1	7500										7500	55500
A370.B8	ASPEN	Air blower			1	657800										657800	917000
A370.B9	ASPEN	Heater dryer air feed		304SS	1	12700										12700	77700
A370.EVAP1	ASPEN	Evaporator		304SS	1	47800										47800	193800
A370.EVAP2	ASPEN	Evaporator		304SS	1	45100										45100	214700
A370.EVP3	ASPEN	Evaporator		304SS	1	30900										30900	144300
A370.NB9	ASPEN	Dryer feed pump		304SS	1	4900	2018									4900	39700
A370.SPRYDAX	ASPEN	Spray dryer		304SS	1	29400										29400	37700
																Area 370	**$1 680 400**
A700.B2	ASPEN	Flash condenser		304SS	1	36400										36400	120100
A700.B2	ASPEN	Vapour condenser		304SS	1	34200										34200	206300
A700.B4	ASPEN	Vapour condenser		304SS	1	108800										108800	372100
																Area 700	**$698 500**
B100.B1	ASPEN	Clear press water pump		304SS		5700	2018									5700	43000
B100.B9	ASPEN	BCS pump		304SS		4900	2018									4900	33300
B100.CNTRFPL	ASPEN	Solid liquid separator	17.2	304SS	1	918400	2018	Mass flow	19698							918400	1425000

B100.DRYBSGPD	ASPEN	Spray drier	304SS	1	42000	2018		42000	210200
B100.EVAP1	ASPEN	Evaporator	304SS		38200	2018		38200	165800
B100.EVAP2	ASPEN	Evaporator	304SS		39700	2018		39700	190700
B100.EVP3	ASPEN	Evaporator	304SS		27400	2018		27400	139800
								Area B100	**$ 2 207 800**
									$18 324 279

6.8.3 Equipment list for scenario C

Table 6A-23 Equipment list for Scenario C: Xylitol and XOS production

Equipment Title	Source	Description	kW	Matel	Qty	$	Year of Quote	Scaling Variable	Scaling Val	Units	Scaling Exp	Inst Factor	New Val	Size Ratio	Scaled P cost	Purch Cost in PY	Inst Cost in PY
	NREL	BSG screw conw	8.5	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	21770	0.760	16064.	18563.6	$31 558
A100.PLPOMP	ASPEN	Liquid press fraction pump				6100	2018									6100	53700
A100.SCPR	Alibaba	Screw press HDL-304	83	304SS	3	46000	2018	dry mass flow	4770	kg/h	0.8	1.5	4770	1	46000	138000	$207 000
															Area 100 Total installed cost		**$292 258**
A200.B1	ASPEN	Heat exch cellulignin			1	9800	2018									9800	60400
A200.B13	ASPEN	Heat exch flash/hydrolysate out			1	10200	2018									10200	60800
A200.B4	ASPEN	Reactor flash tank			1	27400	2018									27400	148900
A200.B6	ASPEN	Hydrolysate pump out			1	5900	2018									5900	51500
A200.B8	ASPEN	Centrifuge cellulignin	14.3		1	688800	2018									688800	1068700
	NREL	Cellulignin screw conveyor	1.7	304SS	1	20000	2009	mass flow	28630	kg/h	0.8	1.7	4450	0.155	4510.	5212.7	8861.6
A200.SREAC	NREL	Acid hydrolysis reactor 1 h 25m3		316SS	3	203000	2009	mass flow	264116	kg/h	0.7	2	18985	0.072	32146	111442	222885
	NREL	Tank agitators x3 75hp	15.0	SS	3	90000	2009	mass flow	264118	kg/h	0.5	1.5	18985	0.072	24130	83651	125477
	NREL	Sulfuric acid tank 24 hr resi		plastic	1	6210	2009	mass flow	1984	kh/h	0.7	0.4	335.5	0.169	1790	2068	827
	NREL	Sulfuric acid pump 8 GPM 245 FT		316SS	1	8000	2009	mass flow	3720	kh/h	0.8	2.3	100.00	0.027	443	512	1178
															Area 200 Total installed cost		**$1 748 884**
A300.B3	ASPEN	hydrolysate to detox pump			1	5500	2018									5500	40300
A300.B4	ASPEN	heat loss out			1	8400	2018									8400	56600
A300.B5	ASPEN	pump hydrolysate out			1	5500	2018									5500	42700
	NREL	Lime screw convev				20000	2009	mass flow	28630	kg/h	0.8	1.7	171	0.006	333	384	1000
A300.DETOXTAN	NREL	1 hr 10m3		304SS	1	236000	2009	mas flow	410369	kg/h	0.7	2	9095.9	0.02	16401	18953	37907
Detox agitator	NREL	Agitator 10 Hp lotus	0.75	SS	1	21900	2009	mass flow	410369	kg/h	0.7	2	9095.9	0.02	1522	1759	3518
A300.EVP1	ASPEN	evaporator			1	58000	2018									58000	263700

A300.EVP2	ASPEN	evaporator			1	45100	2018								45100	227100
A300.GYPSFIL	ASPEN	Ryde DNY Rotary vacuum drum filter dewatering belt press	13	304SS	5	50000	2018	mass flow	450	kg/h	0.8	1.7	2067.2	1 50000	250000	425000
															Area 300 Total installed cost	**$1 097 824**
A350.B1	ASPEN	Nanofiltration membrane			1	3000	2005	m2	30	m2	1	1.5	263	8.8 26333	33921	50881
A350.B10	ASPEN	Pump dilution water diafiltration				6400	2018								6400	54000
A350.B11	ASPEN	Pump nanofiltration				21800	2018								21800	62800
A350.B15	ASPEN	Pump diafiltration				6500	2018								6500	54100
A350.B17	ASPEN	Heatexch cooler diafiltration				13000	2018								13000	69700
A350.B2	ASPEN	Feed pump enzymatic hydrolysis				5500	2018								5500	42700
A350.B3	ASPEN	Outlet enzymatic hydrolysis				5600	2018								5600	42900
A350.B4	ASPEN	Heatexch oligos out				7700	2018								7700	47500
A350.B5	ASPEN	Pump recycle ultrafiltration				4900	2018								4900	39700
A350.B6	ASPEN	Pump nanofiltration				22500	2018								22500	63700
A350.B7	ASPEN	Enzyme AML pump				4900	2018								4900	29000
A350.CONMON	ASPEN	Nanofiltration membrane			1	3000	2005	m2	30	m2	1	1.5	377	12.6 37720	48589.1	72884
A350.CONOLG	ASPEN	Nanofiltration membrane			1	3000	2005	m2	30	m2	1	1.5	189	6.3 18854	24286.5	36430
A350.DIACV	ASPEN	Diafiltration membrane			1	3000	2005	m2	30	m2	1	1.5	475	15.8 47500	61185.9	91779
A350.ENZRHAML	NREL	Enzyme AML reactor		304SS	1	480000	2009	volume	1000	m3	0.7	2	400	0.4 25274	292069	584138
	NREL	Agitator	6	316SS	1	11000	2009		1		1	1.5	1	1 11000	12711	19067
A350.RECULTR	ASPEN	Ultrafiltration membrane			1	3000	2005	m2	30	m2	1	1.5	268	8.9 26777	34492.2	51738
A350.RVDIA	ASPEN	Diafiltration membrane			1	3000	2005	m2	30	m2	1	1.5	52	1.7 5222.9	6727.8	10092
															Area 350	**$1 423 108**
A360.B1	ASPEN	Pump washing 2		304SS		4900	2018								4900	33300
A360.B2	ASPEN	Transfer pump		304SS		5500	2018								5500	42700
A360.B3	ASPEN	Pump hydrolysate out		304SS		6700	2018								6700	44100
A360.B4	ASPEN	Pump washing 1		304SS		4900	2018								4900	33300
		Resin			1	4.7	2003		1 925	kg		1.1		9048	13574	14931
A360.GAMBER 1	ASPEN	Amberlite IRA 400 bed		304SS		289600	2018	flowrate	7430	kg/h	0.7	1.5	8072	1.09	289600	784385

A360.GAMBER 1B	ASPEN	Amberlite IRA 400 bed		304SS		289600	2018	flowrate	7430	kg/h			7397	1.00		289600	722000
A360.GAMBER 2	ASPEN	Amberlite 200		304SS		289600	2018	flowrate	7430	kg/h		2.49				289600	722000
																Area 360	$ 2 396 716
A370.B6	ASPEN	Evaporator feed pump		304SS		5500	2018									5500	42700
A370.B8	ASPEN	Air blower				637400	2018									637400	822000
A370.B9	ASPEN	Heater dryer air feed		304SS		12700	2018									12700	77700
A370.EVAP1	ASPEN	Evaporator		304SS		30900	2018									30900	144300
A370.EVAP2	ASPEN	Evaporator		304SS		27400	2018									27400	139800
A370.EVP3	ASPEN	Evaporator		304SS		27400	2018									27400	139800
A370.NB9	ASPEN	Dryer feed pump		304SS		4900	2018									4900	39700
A370.SPRYDAX	ASPEN	Spray dryer		304SS		25400	2018									25400	32800
																Area 370	**$1 438 800**
A400.B1	ASPEN	Pump out section A500		304SS	1	4900	2018									4900	39700
A400.B2	ASPEN	Holding flash tank		304SS	1	27400	2018									27400	146900
A400.B4	ASPEN	Fermenter flash tank		304SS	1	33700	2018									33700	176000
A400.B5	ASPEN	Condenser evaporator		304SS	1	9700	2018									9700	59700
A400.B6	ASPEN	Fermenter Broth pump out		304SS	1	4900	2018									4900	39700
A400.EVAPXYL	ASPEN	Evaporator		304SS	1	42100	2018									42100	147100
A400.EXHFERM	ASPEN	Heatexch cooler fermenter		304SS	1	9700	2018									9700	59700
A400.XYLCENT	ASPEN	Centrifuge biomass	2.8	304SS	1	128200	2018									128200	199400
	NREL	Seed fermenter 100l		304SS	2	37700	2009		1		0.7	1.8	1	1	37700	87131	156836
	NREL	Seed fermenter agitator	5.625	SS	1	26000	2009	mass flow	1		0.5	1.5	1	1	26000	30045	45068
	NREL	Seed fermenter pump	0.75	304SS	1	8200	2009	mass flow	43149		0.8	2.3	3341	0	1059	1224	2815
A400.XYLFERM	NREL	Fermenter 60h 40m3		304SS	6	844000	2018	volume	4000	m3	0.7	2	40	0.01	33600.2	201601.5	403203
																Area 400	**$1 476 121**
A500.ARBEVAP	ASPEN	Evaporator		304SS	1	27400	2018									27400	146900
A500.B1	ASPEN	Fresh water pump SMB		304SS	1	5700	2018									5700	47200
		Resin			1	4.7	2003		248	kg		1.1			1165.6	1748.69	1923.6

A500.B2	ASPEN	IE desalting column		304SS	1	289600	2018	flow rate	7430	kg/h	0.7	2.49	3270	0.440108	163038.4	163038	406470
A500.B9	ASPEN	Feed pump SMB		304SS	1	5600	2018									5600	34000
A500.CHRSEP	NREL	SMB 9 zone incl resin			1	3120000	1997	feed flow rate l/min	260	l/min	0.8	1.5	15.53	0.1	327482	511008	766512
	Wan	SMB 5 zone			1	1000000	2003	feed flow rate l/min	189	l/min	0.8	1.5	15.53	0.1	135470		
A500.EVAPCHR	ASPEN	Evaporator		304SS	1	27400	2018									27400	146900
A500.HXCHRSEP	ASPEN	Heat exch waste heat SMB			1	14900	2018									14900	71600
		Activated carbon			1	0.9	2013		99	kg		1.1			89.1	94.7227	104.2
A500.MF	ASPEN	Activated carbon bed		304SS	1	289600	2018	flow rate	7430	kg/h	0.7	2.49	3240	0.436	161990	161990	403856
																Area 500	**$2 025 465**
A600.B1	ASPEN	Feed pump evaporator		304SS		5100	2018		4278							5100	40000
A600.B13	ASPEN	Centrifuge 1	0.37	304SS		128200	2018		427							128200	199400
A600.B17	ASPEN	Recycle pump		304SS		4900	2018									4900	31500
A600.B3	ASPEN	Centrifuge 2	0.15	304SS		128200	2018		169							128200	199400
A600.B7	ASPEN	Crystalliser feed pump		304SS		4900	2018									4900	31500
A600.CRDRIER	ASPEN	Crystal dryer		304SS		27700	2018									27700	162400
A600.CREVAP	ASPEN	Second stage evaporator		304SS		33700	2018									33700	168400
A600.DEXFL2	ASPEN	Heat exch vapour condenser		304SS		7700	2018									7700	44000
A600.FLEVB1	ASPEN	Evaporator		304SS		27300	2018									27300	129500
A600.FLEVB2	ASPEN	Evaporator		304SS		30900	2018									30900	144300
A600.WASHER	ASPEN	Drum washer		CS		93800	2018									93800	155100
A600.XYLCRT1	ASPEN	Crystalliser	26	304SS		58100	2018									58100	97600
A600.XYLCRT2	ASPEN	Crystalliser	8.5	304SS		33700	2018									33700	61100
																Area 600	**$1 464 200**
A700.B1	ASPEN	Flash air separator		304SS		62300	2018									62300	273100
A700.B2	ASPEN	Flash condenser		304SS		124600	2018									124600	416200
A700.B4	ASPEN	Vapour condenser		304SS		65000	2018									65000	172200
																Area 700	**$861 500**
B100.B1	ASPEN	Clear press water pump		304SS		5700	2018									5700	43000

B100.B9	ASPEN	BCS pump		304SS	4900	2018			4900	33300
B100.CNTRFPL	ASPEN	Solid liquid separator	17.2	304SS	918400	2018	Mass flow	19698	918400	1425000
B100.DRYBSG PD	ASPEN	Spray drier		304SS	42000	2018			42000	210200
B100.EVAP1	ASPEN	Evaporator		304SS	38200	2018			38200	165800
B100.EVAP2	ASPEN	Evaporator		304SS	39700	2018			39700	190700
B100.EVP3	ASPEN	Evaporator		304SS	27400	2018			27400	139800
									Area B10(	**$ 2 207 800.00**
										$16 432 678

6.9 Discounted cash flow analysis

6.9.1 DCF analysis for Scenario A: Xylitol production

Table 6A-24 Assumptions for cash flow analysis Scenario A

Assumptions	Value		Land Requirement	
Fixed Capital Investment	$35 112 736	$245 789	132	Acres
General Plant	$35 112 736		$0	/acre
Equity	100%			
Loan Interest	8.0%			
Loan Term, years	10			
Annual Loan Payment	$0			
Periodic expenses	KG			
Activated carbon (3 yr life)	527	kg	$ 900.	$/t
IE resin (5 yr life)	19858	kg	$ 4.7	$/kg
Membrane replacement (2 yr cost)	0	m2	$ 66.67	/m2
Working Capital (% of FCI)	5.00%			
Salvage Value		Depreciation	Straight line	
General Plant	$7 022 547	Salvaging value	20%	
		% of initial price		
Plant life	25	years		
Construction Period (Years)	0			
% Spent in Year -2	0%			
% Spent in Year -1	0%			
% Spent in Year 0	100%			
Start-up Time (Years)	0.00			
Production/Feedstock use	0%			
Variable Costs (% of Normal)	0%			
Fixed Cost (% of Normal)	0%			
Income Tax Rate	28.00%			
Xylitol Production Rate (t/y)	2 748			
Cost Year for Analysis	2018			
Inflation Rate	5.7%			
Discount Rate (Internal Rate of Return [IRR])	9.7%			
Xylitol Selling Price ($/t)	$4 500			
IRR	12.3%			
Net Present Value	$6 766 777			

Total Capital Investment

Process Area			Purchased Cost	Installed Cost
			$ -	$ -
Area A100: Feedstock preparing			$ 162 664	$ 292 258
Area B100: Press liquid upgrading			$ 1 076 300	$ 2 207 800
Area A200: Hydrothermal treatment			$ 3 942 891	$ 6 277 568
Area A300: Neutralisation			$ 221 967	$ 937 523
Area A400: Fermentation			$ 1 095 049	$ 2 355 540
Area A500: Purification			$ 1 424 201	$ 3 043 915
Area A600: Crystallisation			$ 666 800	$ 1 641 500
Area A700: Wastewater			$ 69 400	$ 257 300
Area A800: Storage (5 % of ISBL)			$ 436 625	$ 849 874
Area A900: Utilities (6.5% of ISBL)			$ 567 612	$ 1 104 836
Totals			$ 9 663 508	$ 18 968 114
Warehouse	4.0%	of ISBL		$ 680 536
Site Development	9.0%	of ISBL		$ 1 531 206
Additional Piping	4.5%	of ISBL		$ 765 603
Total Direct Costs (TDC)				$ 21 945 460
Prorate able Expenses	10.0%	of TDC		$ 2 194 546
Field Expenses	10.0%	of TDC		$ 2 194 546
Home Office & Construction Fee	20.0%	of TDC		$ 4 389 092
Project Contingency	10.0%	of TDC		$ 2 194 546
Other Costs (Start-Up, Permits, etc.)	10.0%	of TDC		$ 2 194 546
Total Indirect Costs				$ 13 167 276
Fixed Capital Investment (FCI)				$ 35 112 736
South Africa Location Factor (LF)				1.0
Corrected Fixed Capital Investment (FCI)				$ 35 112 736
Working Capital	5.0%	of FCI		$ 1 755 637
Land				$ -
Corrected Total Capital Investment (TCI)				$ 36 868 373
Lang Factor (FCI/Purchased Equip Cost)				
TCI per annual tonne xylitol				$12779
MM$ TCI per day tonne xylitol				$4.26/t

Table 6A-25 Variable and fixed operating cost

Operating Cost Analysis											
Operating h/y	8 000						Feedstock cost	$ 10	/ tonne		
Variable Operating Costs											
Area	**Costing Code**	**Raw Material**	**Stream No.**	**kg/hr**		**Quoted Price ($ / t)**	**Year of Price Quote**	**2018 cost $ /ton**		**$/hour**	**MM $ /Year 2018**
				Raw Materials							
A100	BSG dewatering	Feedstock	BSG	41 667	kg/h	10	2018	10		416.67	3.33
A200	Hydrothermal treatment	Sulfuric Acid, 98%	H2SO4	114	kg/h	50	2009	57.8		6.58	0.05
A200	Hydrothermal treatment	Wash water	Water	8 661	m3	1.20	2016	1.3		11.26	0.09
A300	Neutralisation	Lime	Lime	89	kg/h	120	2009	138.7		12.34	0.10
A400	Fermentation	Oxygen	O2	63	kg/h	300	2015	318.9		20.09	0.16
A400	Fermentation	Yeast	Biomass	116	kg/h	350	2016	379.1		43.98	0.35
A500	Purification	Water eluent	Water	30 197	kg/h	1	2016	1.3		39.25	0.31
A500	Purification	Boiler water	Steam	1 004	kg/h	3	2016	3.2		3.26	0.03
A600	Crystallisation	Wash water	Water	33	kg/h	1	2016	1.3		0.04	0.00
		Subtotal								**553.5**	**4.43**
				Waste Streams							
A300	Neutralisation	Gypsum	CaSO4	465	kg/h	7.5	2018	7.50		3.49	0.03
A300	Neutralisation	AC impurities	AC out	485	kg/h	7.5	2018	7.50		3.64	0.03
A500	Purification	AC impurities	AC out	109	kg/h	7.5	2018	7.50		0.82	0.01
A500	Purification	IE impurities	IE salts	133	kg/h	7.5	2018	7.50		1.00	0.01
A500	Purification	Waste water	CHDWW	20 649	m3	0.8	2003	1.20		24.78	0.20
A700	Waste water		Condensate	52 997	m3	0.8	2003	1.20		63.61	0.51
		Subtotal								**97.3**	**0.78**
				Utilities							
All	Steam	Steam	Steam LP	18 313	kg/h	3.000	2013	3.19		58.41	0.4672
A200	Steam	Steam	Steam HP	337	kg/h	8.000	2013	8.50		2.86	0.0229
All	Electricity	Electricity		1 205	kW	0.200	2018	0.20		240.96	1.9276
All	Cooling water	Cooling water		2 576	kg/h	0.020	2003	0.03		77.31	0.6185
		Subtotal								**379.53**	**3.04**

Recurring operating expense										
A300	Neutralisation	Activated carbon bed	AC regeneration	296	kg	0.020	2003	0.03	0.01	0.0001
A500	Purification	Activated carbon bed	AC regeneration	231	kg	0.020	2003	0.03	0.01	0.0001
A500	Purification	Ion exchange bed	IE regeneration	578	kg	0.196	2003	0.29	0.17	0.0014
A500	Purification	SMB chromatographic	IE regeneration	19 280	kg	0.196	2003	0.29	5.67	0.0454
		Subtotal							**5.86**	**0.05**
By-Products and Credits										
B100	Press liquid upgrade	BDP	BDP	1 345	kg/h	350	2018	350.0	470.78	3.77
B100	Press liquid upgrade	BCS	BCS	1 119	kg/h	10	2018	10.0	11.19	0.09
A200	Hydrothermal treatment	Cellulignin	Cellulignin	4 450	kg/h	8	2018	8.0	35.60	0.28
A400	Fermentation	Spent yeast	Biomass	189	kg/h	150	2018	150.0	28.28	0.23
A500	Purification	Arabinose syrup	Aracon	430	kg/h	400	2018	400.0	171.96	1.38
A600	Crystallisation	Spent mother liquid	CRA	8	kg/h	150	2018	150.0	1.13	0.01
		Subtotal							**718.94**	**5.75**
		Total Variable Operating Costs							**-62.28**	**2.54**

Fixed Operating Costs							
	Position	**Salary**	**Year of salary quote**	**2018 Salary**	**# Required**	**Total**	**MM $ /Year 2018**
	Labour & Supervision						
	Plant Manager	147000	2009	169 871	1	169 871	
	Plant Engineer	70000	2009	80 891	1	80 891	
	Maintenance Supr	57000	2009	65 868	1	65 868	
	Maintenance Tech	40000	2009	46 223	6	277 340	
	Lab Manager	56000	2009	64 713	1	64 713	
	Lab Technician	40000	2009	46 223	2	92 447	
	Shift Supervisor	48000	2009	55 468	4	221 872	
	Shift Operators	40000	2009	46 223	15	693 351	
	Yard Employees	28000	2009	32 356	3	97 069	
	Clerks & Secretaries	36000	2009	41 601	3	124 803	
	Total Salaries				37	1 888 226	**1.89**
	Labour Burden (90%)					1 699 404	**1.70**
	Other Overhead						
	Maintenance	3.0%	of ISBL			510 402	0.51
	Property Insur. & Tax	0.7%	of FCI			245 789	0.25
	Total Fixed Operating Costs						**4.34**
				ISBL=	$17 013 404		
				FCI=	$35 112 736		
							MM $ /Year 2018
Total Operating Costs							**6.88**

Table 6A-26 Discounted cash flow calculation

DCF Worksheet	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Fixed Capital Investment	35112736												
Land													
Working Capital	1755637												
Xylitol Sales	0	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012
Total Annual Sales	**0**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**
Annual Manufacturing Cost													
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	0	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292
Fixed Operating Costs	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822
Periodic expenses	**0**	**0**	**0**	**0**	**0**	**93333**	**0**	**0**	**0**	**0**	**93333**	**0**	**0**
Total Product Cost	**0**	**6881856**	**6881856**	**6881856**	**6881856**	**6975189**	**6881856**	**6881856**	**6881856**	**6881856**	**6975189**	**6881856**	**6881856**
Annual Depreciation	0	0	0	0	0	0	0	0	0	0	0	0	0
Depreciation Charge	0	7022547	7022547	7022547	7022547	7022547	0	0	0	0	0	0	0
Remaining Value	0	28090189	21067642	14045095	7022547	0	0	0	0	0	0	0	0
Net Revenue	-36868373	-1540392	-1540392	-1540392	-1540392	-1633725	5482155	5482155	5482155	5482155	5388822	5482155	5482155
Losses Forward			-1540392	-3080784	-4621176	-6161569	-7795293	-2313138	0	0	0	0	0
Taxable Income		-1540392	-3080784	-4621176	-6161569	-7795293	-2313138	3169017	5482155	5482155	5388822	5482155	5482155
Income Tax		0	0	0	0	0	0	887325	1535003	1535003	1508870	1535003	1535003
Annual Cash Income	**-36868373**	**5482155**	**5482155**	**5482155**	**5482155**	**5388822**	**5482155**	**4594830**	**3947152**	**3947152**	**3879952**	**3947152**	**3947152**

DCF Worksheet (Cont)	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Fixed Capital Investment													
Land													
Working Capital													
Xylitol Sales	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012	12364012
Total Annual Sales	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**	**12364012**
Annual Manufacturing Cost													
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-795292	-3411086
Fixed Operating Costs	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822	4343822
Periodic expenses	0	0	93333	0	0	0	0	93333	0	0	0	0	93333
Total Product Cost	6881856	6881856	6975189	6881856	6881856	6881856	6881856	6975189	6881856	6881856	6881856	6881856	4359395
Annual Depreciation	0	0	0	0	0	0	0	0	0	0	0	0	0
Depreciation Charge	0	0	0	0	0	0	0	0	0	0	0	0	0
Remaining Value	0	0	0	0	0	0	0	0	0	0	0	0	0
Net Revenue	5482155	5482155	5388822	5482155	5482155	5482155	5482155	5388822	5482155	5482155	5482155	5482155	5388822
Losses Forward	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxable Income	5482155	5482155	5388822	5482155	5482155	5482155	5482155	5388822	5482155	5482155	5482155	5482155	5388822
Income Tax	1535003	1535003	1508870	1535003	1535003	1535003	1535003	1508870	1535003	1535003	1535003	1535003	1508870
Annual Cash Income	3947152	3947152	3879952	3947152	3947152	3947152	3947152	3879952	3947152	3947152	3947152	3947152	3879952

6.9.2 DCF analysis for Scenario B: XOS production

Table 6A-27 Assumptions for cash flow analysis Scenario B

Assumptions	Value			Land Reqm	
Fixed Capital Investment	$37 745 685		$264 220	132	Acres
General Plant	$37 745 685			$0	/acre
Equity	100%				
Loan Interest	8.0%				
Loan Term, years	10				
Annual Loan Payment	$0				
Periodic expenses	KG				
IE resin (5 yr life)	24824	kg		$ 4.7	$/kg
Membrane replacement (2 yr cost)	1665	m2		$ 66.67	/m2
Working Capital (% of FCI)	5.00%				
Salvage Value		Depreciation		Straight line	
General Plant	$7 549 137	Salvaging value % of initial price		20%	
Plant life	25	years			
Construction Period (Years)	0				
% Spent in Year -2	0%				
% Spent in Year -1	0%				
% Spent in Year 0	100%				
Start-up Time (Years)	0.00				
Production/Feedstock use	0%				
Variable Costs (% of Normal)	0%				
Fixed Cost (% of Normal)	0%				
Income Tax Rate	28.00%				
XOS Production Rate (t/y)	**5 569**				
Cost Year for Analysis	2018				
Inflation Rate	5.7%				
Discount Rate (Internal Rate of Return [IRR])	**9.7%**				
XOS Selling Price ($/t)	**$4 500**				
IRR	**33.0%**				
Net Present Value	**$75 039 329**				

Total Capital Investment

Process Area			Purchased Cost	Installed Cost
			$ -	$ -
Area A100: Feedstock preparing			$ 162 664	$ 292 258
Area B100: Press liquid upgrading			$ 1 076 300	$ 2 207 800
Area A200: Hydrothermal treatment			$ 3 673 987	$ 5 688 800
Area A350: Diafiltration			$ 1 419 023	$ 3 072 815
Area A360: Purification			$ 2 248 493	$ 4 683 706
Area A370: Drying			$ 836 100	$ 1 680 400
Area A700: Wastewater			$ 179 400	$ 698 500
Area A800: Storage (5 % of ISBL)			$ 471 129	$ 895 663
Area A900: Utilities (6.5% of ISBL)			$ 612 468	$ 1 164 362
Totals (Excl. Area 100)			**$ 10 679 563**	**$ 20 384 304**
Warehouse	4.0%	of ISBL		$ 732 971
Site Development	9.0%	of ISBL		$ 1 649 185
Additional Piping	4.5%	of ISBL		$ 824 593
Total Direct Costs (TDC)				**$ 23 591 053**
Pro-rateable Expenses	10.0%	of TDC		$ 2 359 105
Field Expenses	10.0%	of TDC		$ 2 359 105
Home Office & Construction Fee	20.0%	of TDC		$ 4 718 211
Project Contingency	10.0%	of TDC		$ 2 359 105
Other Costs (Start-Up, Permits, etc.)	10.0%	of TDC		$ 2 359 105
Total Indirect Costs				**$ 14 154 632**
Fixed Capital Investment (FCI)				**$ 37 745 685**
South Africa Location Factor (LF)				1.0
Corrected Fixed Capital Investment (FCI)				**$ 37 745 685**
Working Capital	5.0%	of FCI		$ 1 887 284
Land				$ -
Corrected Total Capital Investment (TCI)				**$ 39 632 969**
Lang Factor (FCI/Purchased Equip Cost)				
TCI per annual tonne XOS				$6778
MM$ TCI per daily tonne				$2.26/t

Table 6A-28 Variable and fixed operating cost

Operating Cost Analysis												
Operating Hours/Yr	8 000						Feedstock cost	$ 10	/Metric ton			
Variable Operating Costs												
Area	**Costing Code**	**Raw Material**	**Stream No.**	**kg/hr**		**Quoted Price ($ / t)**	**Year of Price Quote**	**2018 cost $ /ton**			**$/hour**	**MM $ /Year 2018**
				Raw Materials								
A100	BSG dewatering	Feedstock	BSG	41 667	kg/h	10	2018	10			416.67	3.33
A200	Hydrothermal treatment	Wash water	Water	8 900	m3	1.20	2016	1.3			11.57	0.09
A350	Filtration	Enzyme AMG	AMG	2	kg/h	3000	2009	3466.8			6.51	0.05
A351	Filtration	Enzyme EndXyl	ENXL	14	kg/h	8000	2009	9244.7			130.71	1.05
A350	Filtration	Diafiltration water	Water	89 314	m3	1.2	2016	1.3			116.09	0.93
A360	XOS Purification	Wash water	Water	4 066	m3	1.2	2016	1.3			5.29	0.04
		Subtotal									**686.8**	**5.49**
				Waste Streams								
A350	Filtration	Waste water	Waste water	89 957	m3	0.8	2003	1.20			107.97	0.86
A360	XOS Purification	Waste water	Waste water	25 597	m3	0.8	2003	1.20			30.72	0.25
A700	Waste water		Condensate	30 536	m3	0.8	2003	1.20			36.65	0.29
		Subtotal									**175.3**	**1.40**
				Utilities								
All	Steam	Steam	Steam LP	27 629	kg/h	3.000	2013	3.19			88.12	0.7049
A200	Steam	Steam	Steam HP	337		8.000	2013	8.50			2.86	0.0229
All	Electricity	Electricity		836	kW	0.200	2018	0.20			167.24	1.3379
All	Cooling water	Cooling water		1 521 545	kg/h	0.020	2003	0.03			45.65	0.3652
		Subtotal									**303.88**	**2.43**
				Recurring operating expense								
A350	Diafiltration	Membrane expense	Memb cleaning	1 665	m2	8.33	2005	10.73	year		17 869	0.0179
A360	XOS Purification	IRA 400 +200 Amb	IE regen	24 824	kg	0.196	2003	0.29			7.30	0.0584
		Subtotal									**17 877.1**	**0.08**
				By-Products and Credits								
B100	Press liquid upgrade	BDP	BDP	1 345	kg/h	350	2018	350.0			470.78	3.77
B100	Press liquid upgrade	BCS	BCS	1 119	kg/h	10	2018	10.0			11.19	0.09
A200	Hydrothermal treatment	Cellulignin	Cellulignin	4 450	kg/h	8	2018	8.0			35.60	0.28
		Subtotal									**517.57**	**4.14**
		Total Variable Operating Costs									**18 221.7**	**5.26**

Fixed Operating Costs (Cont.)							
	Position	Salary	Year of salary quote	2018 Salary	# Required	Total	MM $ /Year 2018
				Labour & Supervision			
				169			
	Plant Manager	147000	2009	871	1	169 871	
	Plant Engineer	70000	2009	80 891	1	80 891	
	Maintenance Supr	57000	2009	65 868	1	65 868	
	Maintenance Tech	40000	2009	46 223	6	277 340	
	Lab Manager	56000	2009	64 713	1	64 713	
	Lab Technician	40000	2009	46 223	2	92 447	
	Shift Supervisor	48000	2009	55 468	4	221 872	
	Shift Operators	40000	2009	46 223	8	369 787	
	Yard Employees	28000	2009	32 356	3	97 069	
	Clerks & Secretaries	36000	2009	41 601	3	124 803	
	Total Salaries				30	1 564 663	**1.56**
	Labour Burden (90%)					1 408 196	**1.41**
				Other Overhead			
	Maintenance	3.0%	of ISBL			549 728	0.55
	Property Insur. & Tax	0.7%	of FCI			264 220	0.26
	Total Fixed Operating Costs						**3.79**
			ISBL=		$18 324 279		
			FCI=		$37 745 685		
							MM $ /Year 2018
Total Operating Costs							**9.05**

Table 6A-29 Discounted cash flow calculation

DCF Worksheet	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	0	1	2	3	4	5	6	7	8	9	10	11	12
Fixed Capital Investment	37745685												
Land	0												
Working Capital	1887284												
XOS Sales	0	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302
Total Annual Sales	**0**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**
Annual Manufacturing Cost	0	0	0	0	0	0	0	0	0	0	0	0	0
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	0	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763
Fixed Operating Costs	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807
Periodic expenses	0	0	110982	0	110982	116673	110982	0	110982	0	227655	0	110982
Total Product Cost	**0**	**9050897**	**9161879**	**9050897**	**9161879**	**9167570**	**9161879**	**9050897**	**9161879**	**9050897**	**9278552**	**9050897**	**9161879**
Annual Depreciation													
Depreciation Charge	0	7549137	7549137	7549137	7549137	7549137	0	0	0	0	0	0	0
Remaining Value	0	30196548	22647411	15098274	7549137	0	0	0	0	0	0	0	0
Net Revenue	-39632969	8458268	8347286	8458268	8347286	8341595	15896423	16007405	15896423	16007405	15779750	16007405	15896423
Losses Forward			0	0	0	0	0	0	0	0	0	0	0
Taxable Income		8458268	8347286	8458268	8347286	8341595	15896423	16007405	15896423	16007405	15779750	16007405	15896423
Income Tax		2368315	2337240	2368315	2337240	2335647	4450998	4482073	4450998	4482073	4418330	4482073	4450998
Annual Cash Income	**-39632969**	**13639090**	**13559183**	**13639090**	**13559183**	**13555085**	**11445425**	**11525332**	**11445425**	**11525332**	**11361420**	**11525332**	**11445425**

DCF Worksheet (Cont.)	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	13	14	15	16	17	18	19	20	21	22	23	24	25
Fixed Capital Investment													
Land													
Working Capital													
XOS Sales	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302	25058302
Total Annual Sales	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**	**25058302**
Annual Manufacturing Cost													
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763	1930763
Fixed Operating Costs	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807	3786807
Periodic expenses	0	110982	116673	110982	0	110982	0	227655	0	110982	0	110982	116673
Total Product Cost	**9050897**	**9161879**	**9167570**	**9161879**	**9050897**	**9161879**	**9050897**	**9278552**	**9050897**	**9161879**	**9050897**	**9161879**	**9167570**
Annual Depreciation													
Depreciation Charge	0	0	0	0	0	0	0	0	0	0	0	0	0
Remaining Value	0	0	0	0	0	0	0	0	0	0	0	0	0
Net Revenue	16007405	15896423	15890732	15896423	16007405	15896423	16007405	15779750	16007405	15896423	16007405	15896423	15890732
Losses Forward	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxable Income	16007405	15896423	15890732	15896423	16007405	15896423	16007405	15779750	16007405	15896423	16007405	15896423	15890732
Income Tax	4482073	4450998	4449405	4450998	4482073	4450998	4482073	4418330	4482073	4450998	4482073	4450998	4449405
Annual Cash Income	**11525332**	**11445425**	**11441327**	**11445425**	**11525332**	**11445425**	**11525332**	**11361420**	**11525332**	**11445425**	**11525332**	**11445425**	**11441327**

6.9.3 DCF analysis for Scenario C: Xylitol and XOS production

Table 6A-30 Assumptions for cash flow analysis Scenario C

Assumptions	Value				
			1.00		
			Land Requirent		
Fixed Capital Investment	$33 918 377	$237 393	132	Acres	
General Plant	$33 918 377		$0	/acre	
Equity	100%				
Loan Interest	8.0%				
Loan Term, years	10				
Annual Loan Payment	$0				
Periodic expenses	KG				
Activated carbon (3 yr life)	99	kg	$ 900	$/t	
IE resin (5 yr life)	10955	kg	$ 4.70	$/kg	
Membrane replace (2 yr cost)	1624	m2	$ 66.67	/m2	
Working Capital (% of FCI)	5.00%				
Salvage Value		Depreciation method	Straight line		
General Plant	$6 782 644	Salvaging value %l int st	20%		
Plant life	25	years			
Construction Period (Years)	0				
% Spent in Year -2	0%				
% Spent in Year -1	0%				
% Spent in Year 0	100%				
Start-up Time (Years)	0.00				
Production/Feedstock use	0%				
Variable Costs (% of Normal)	0%				
Fixed Cost (% of Normal)	0%				
Income Tax Rate	28.00%				
Xylitol Production Rate (t/y)	**1 169**				
XOS Production Rate (t/y)	**3 840**				
Cost Year for Analysis	2018				
Inflation Rate	5.7%				
Discount Rate (Internal Rate of Return [IRR])	**9.7%**				
Selling Price Xylitol ($/t)	**$4 500**				
Selling Price XOS($/t)	**$4 500**				
IRR	**36.5%**				
Net Present Value	**$78 465 867**				

Total Capital Investment

Process Area			Purchased Cost	Installed Cost
			$ -	$ -
Area A100: Feedstock preparing			$ 162 664	$ 292 258
Area B100: Press liquid upgrading			$ 1 076 300	$ 2 207 800
Area A200: Hydrothermal treatment			$ 944 987	$ 1 749 529
Area A300: Neutralisation			$ 393 313	$ 1 097 824
Area A350: Filtration			$ 612 782	$ 1 423 108
Area A360: Purification			$ 904 374	$ 2 396 716
Area A370: Drying			$ 771 600	$ 1 438 800
Area A400: Fermentation			$ 580 602	$ 1 476 122
Area A500: Purification			$ 918 879	$ 2 025 465
Area A600: Crystallisation			$ 584 200	$ 1 464 200
Area A700: Wastewater			$ 251 900	$ 861 500
Area A800: Storage (5 % of ISBL)			$ 360 094	$ 821 666
Area A900: Utilities (6.5% of ISBL)			$ 468 122	$ 1 068 166
Totals (Excl. Area 100)			**$ 8 030 101**	**$ 18 323 154**
Warehouse	4.0%	of ISBL		$ 657 333
Site Development	9.0%	of ISBL		$ 1 478 999
Additional Piping	4.5%	of ISBL		$ 739 500
Total Direct Costs (TDC)				**$ 21 198 986**
Pro-rateable Expenses	10.0%	of TDC		$ 2 119 899
Field Expenses	10.0%	of TDC		$ 2 119 899
Home Office & Construction Fee	20.0%	of TDC		$ 4 239 797
Project Contingency	10.0%	of TDC		$ 2 119 899
Other Costs (Start-Up, Permits, etc.)	10.0%	of TDC		$ 2 119 899
Total Indirect Costs				**$ 12 719 391**
Fixed Capital Investment (FCI)				**$ 33 918 377**
South Africa Location Factor (LF)				1.0
Corrected Fixed Capital Investment (FCI)				**$ 33 913 219**
Working Capital	5.0%	of FCI		$ 1 695 661
Land				$ -
Corrected Total Capital Investment (TCI)				**$ 35 614 296**
Lang Factor (FCI/Purchased Equip Cost)				
TCI per annual tonne xylitol				$29001
TCI per annual tonne XOS	$8831			
MM$ TCI per day tonne xylitol				$4.45
MM$ TCI per day tonne XOS				$1.74

Table 6A-31 Variable and fixed operating cost

Operating Cost Analysis

Operating Hours/Yr	8 000		Feedstock cost	$ 10	/Metric ton

Variable Operating Costs

Area	Costing Code	Raw Material	Stream No.	kg/hr		Quoted Price ($ / t)	Year of Price Quote	2018 cost $ /ton	$/hour	MM $ /Year 2018
				Raw Materials						
A100	BSG dewatering	Feedstock	BSG	41 667		10	2018	10	416.67	3.33
A200	Hydrothermal treatment	Sulfuric Acid, 98%	H2SO4	224		50	2009	57.8	12.94	0.10
A200	Hydrothermal treatment	Wash water	Water	8 522	m3	1.20	2016	1.3	11.08	0.09
A300	Neutralisation	Lime	Lime	171		120	2009	138.7	23.71	0.19
A400	Fermentation	Oxygen	O2	29		300	2015	318.9	9.25	0.07
A400	Fermentation	Yeast	Biomass	38	kg	350	2016	379.1	14.41	0.12
A500	Purification	Water eluent	Water	13 653		1	2016	1.3	17.75	0.14
A500	Purification	Boiler water	Steam	449		3	2016	3.2	1.46	0.01
A600	Crystallisation	Wash water	Water	14		1	2016	1.3	0.02	0.00
A350	Filtration	Enzyme AMG	AMG	2		3000	2009	3466.8	7.05	0.06
A350	Filtration	Diafiltration water	Water	23 787	m3	1.2	2016	1.3	30.92	0.25
A360	XOS Purification	Wash water	Water	2 582	m3	1.2	2016	1.3	3.36	0.03
		Subtotal							**548.6**	**4.39**
				Waste Streams						
A300	Neutralisation	Gypsum	CaSO4	524	kg/h	7.5	2018	7.50	3.93	0.03
A500	Purification	AC impurities	AC out	109	kg/h	7.5	2018	7.50	0.82	0.01
A500	Purification	IE impurities	IE salts	133	kg/h	7.5	2018	7.50	1.00	0.01
A500	Purification	Waste water	CHDWW	9 426	m3	0.8	2003	1.20	11.31	0.09
A350	Filtration	Waste water	Waste water	19 897	m3	0.8	2003	1.20	23.88	0.19
A360	XOS Purification	Waste water	Waste water	3 402	m3	0.8	2003	1.20	4.08	0.03
A700	Waste water		Condensate	45 379	m3	0.8	2003	1.20	54.46	0.44
		Subtotal							**99.5**	**0.80**
				Utilities						
All	Steam	Steam	Steam LP	23 110	kg/h	3.000	2013	3.19	73.70	0.5896
All	Electricity	Electricity		629	kW	0.200	2018	0.20	125.72	1.0057
All	Cooling water	Cooling water		2 233 931	kg/h	0.020	2003	0.03	67.03	0.5362
		Subtotal							**266.45**	**2.13**

Variable Operating Costs (Cont.)											
Recurring operating expense											
A500	Purification	Activated carbon bed	AC regen	99	kg	0.020	2003	0.03		0.00	0.0000
A500	Purification	Ion exchange bed	IE regen	248	kg	0.196	2003	0.29		0.07	0.0006
A500	Purification	SMB chromatographic	IE regen	8 782	kg	0.196	2003	0.29		2.58	0.0207
A350	Filtration	Membrane expense	Membr clean	1 624	m2	8.33	2005	10.73	year	2.18	0.0174
A360	XOS Purification	IRA 400 +200 Amb	IE regen	1 925	kg	0.196	2003	0.29		0.57	0.0045
		Subtotal								**5.4**	**0.04**
By-Products and Credits											
B100	Press liquid upgrade	BDP	BDP	1 345	kg/h	100	2018	350.0		470.78	3.77
B100	Press liquid upgrade	BCS	BCS	1 119	kg/h	10	2018	10.0		11.19	0.09
A200	Hydrothermal treatment	Cellulignin	Cellulignin	4 261	kg/h	8	2018	8.0		34.09	0.27
A400	Fermentation	Spent yeast	Biomass	71	kg/h	150	2018	150.0		10.65	0.09
A500	Purification	Arabinose syrup	Aracon	399	kg/h	400	2018	400.0		159.66	1.28
A600	Crystallisation	Spent mother liquid	CRA	2	kg/h	150	2018	150.0		0.37	0.00
		Subtotal								**686.73**	**5.49**
		Total Variable Operating Costs								**-33.3**	**1.87**

Fixed Operating Costs							
	Position	**Salary**	**Year of quote**	**2018 Salary**	**# Required**	**Total**	**MM $ /Year 2018**
Labour & Supervision							
	Plant Manager	147000	2009	169 871	1	169 871	
	Plant Engineer	70000	2009	80 891	2	161 782	
	Maintenance Supr	57000	2009	65 868	1	65 868	
	Maintenance Tech	40000	2009	46 223	8	369 787	
	Lab Manager	56000	2009	64 713	1	64 713	
	Lab Technician	40000	2009	46 223	2	92 447	
	Shift Supervisor	48000	2009	55 468	4	221 872	
	Shift Operators	40000	2009	46 223	15	693 351	
	Yard Employees	28000	2009	32 356	3	97 069	
	Clerks & Secretaries	36000	2009	41 601	3	124 803	
	Total Salaries				40	2 061 564	**2.06**
	Labour Burden (90%)					1 855 408	**1.86**
Other Overhead							
	Maintenance	3.0%	of ISBL			492 980	0.49
	Property Insur. & Tax	0.7%	of FCI			237 393	0.24
	Total Fixed Operating Costs						**4.65**
				ISBL=	$16 432 678		
				FCI=	$33 913 219		

	MM $ /Year 2018
Operating Costs	**6.51**

Table 6A-32 Discounted cash flow calculation

	XOS	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
DCF Worksheet	Xylitol	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	0	1	2	3	4	5	6	7	8	9	10	11	12
Fixed Capital Investment	33918377												
Working Capital	1695661												
Xylitol Sales	0	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132
XOS Sales	0	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993
Total Annual Sales	**0**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**
Annual Manufacturing Cost													
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	0	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864
Fixed Operating Costs	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400
Periodic expenses			108272		108272	51489	108272		108272		159761		108272
Total Product Cost	**0**	**6512863**	**6621135**	**6512863**	**6621135**	**6564351**	**6621135**	**6512863**	**6621135**	**6512863**	**6672624**	**6512863**	**6621135**
Annual Depreciation													
Depreciation Charge	0	6783675	6783675	6783675	6783675	6783675	0	0	0	0	0	0	0
Remaining Value	0	27134702	20351026	13567351	6783675	0	0	0	0	0	0	0	0
Net Revenue	**-35614296**	**9245587**	**9137314**	**9245587**	**9137314**	**9194098**	**15920990**	**16029262**	**15920990**	**16029262**	**15869501**	**16029262**	**15920990**
Losses Forward			0	0	0	0	0	0	0	0	0	0	0
Taxable Income		9245587	9137314	9245587	9137314	9194098	15920990	16029262	15920990	16029262	15869501	16029262	15920990
Income Tax		2588764	2558448	2588764	2558448	2574347	4457877	4488193	4457877	4488193	4443460	4488193	4457877
Annual Cash Income	**-35614296**	**13440498**	**13362542**	**13440498**	**13362542**	**13403426**	**11463113**	**11541069**	**11463113**	**11541069**	**11426041**	**11541069**	**11463113**

(Cont.)	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
DCFR Worksheet	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500	4500
Year	13	14	15	16	17	18	19	20	21	22	23	24	25
Fixed Capital Investment													
Working Capital													
Xylitol Sales	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132	5262132
XOS Sales	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993	17279993
Total Annual Sales	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**	**22542125**
Annual Manufacturing Cost													
Feedstock Price ($/ ton)	10	10	10	10	10	10	10	10	10	10	10	10	10
Feedstock cost	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327	3333327
Other Variable Costs	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1467864	-1720122
Fixed Operating Costs	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647400	4647345
Periodic expenses		108272	51489	108272		108272		159761		108272		108272	51489
Total Product Cost	**6512863**	**6621135**	**6564351**	**6621135**	**6512863**	**6621135**	**6512863**	**6672624**	**6512863**	**6621135**	**6512863**	**6621135**	**6312038**
Net Revenue	16029262	15920990	15977774	15920990	16029262	15920990	16029262	15869501	16029262	15920990	16029262	15920990	15977774
Losses Forward	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxable Income	16029262	15920990	15977774	15920990	16029262	15920990	16029262	15869501	16029262	15920990	16029262	15920990	15977774
Income Tax	4488193	4457877	4473777	4457877	4488193	4457877	4488193	4443460	4488193	4457877	4488193	4457877	4473777
Annual Cash Income	**11541069**	**11463113**	**11503997**	**11463113**	**11541069**	**11463113**	**11541069**	**11426041**	**11541069**	**11463113**	**11541069**	**11463113**	**11503997**

6.9.4 Economic assessment

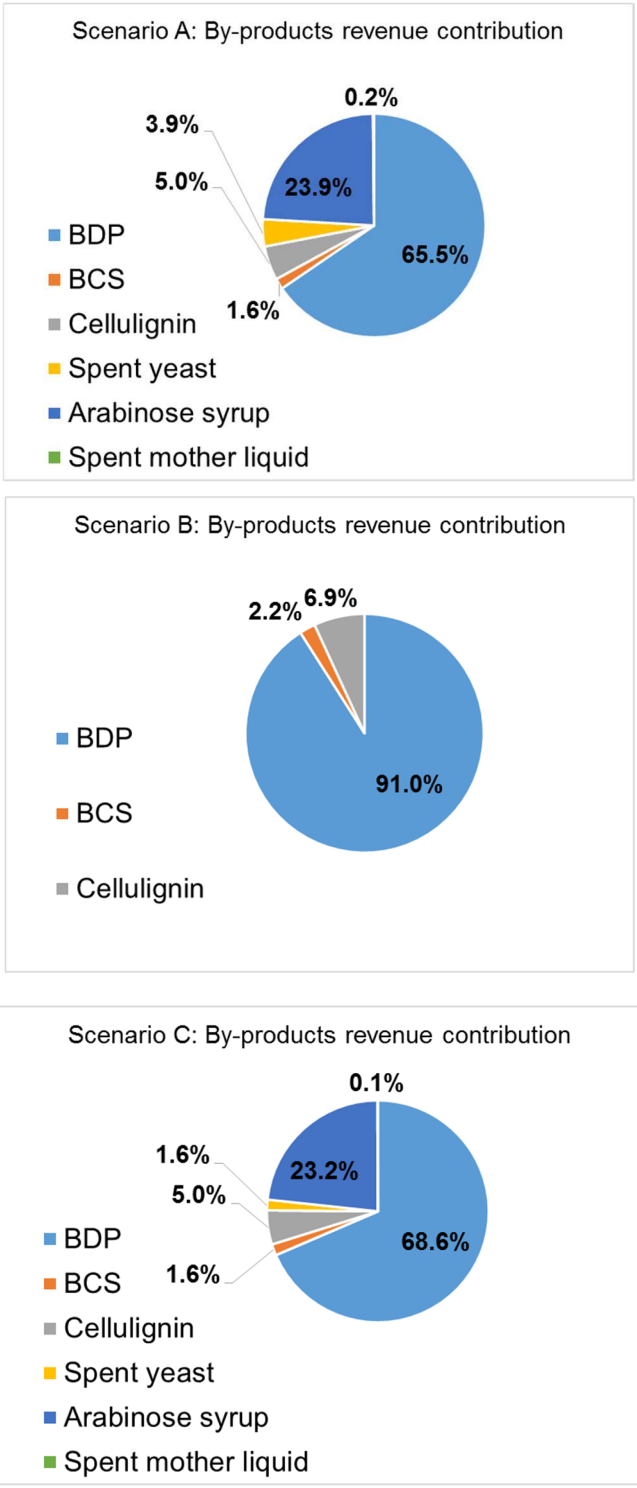

Figure 6A-25 By-product revenue contribution per scenario

7. Conclusions and recommendations

7.1 Conclusions

A. Objective #1: HTT techniques for fractionation of BSG in an energy efficient manner, by using higher dry matter contents

Screw press dewatering of BSG for increased solids loading

1. **<u>Screw press dewatering is an efficient method for biomass moisture reduction for increased solids loadings in HTT</u>**

   Mechanical dewatering of biomass using a screw press can be an efficient method to increase solids loading. Three levels of moisture contents were obtained for the study using raw BSG with 15% dry matter which was pressed to,

   (i) a 25% dry matter (*ca* 47% less moisture) as in **Chapter 4** for a Weiss BSG and

   (ii) and additional 32% dry matter (another *ca* 29% less moisture) **Chapter 5** with Weiss and Malt BSG's

   This study showed that the application of a screw press for dewatering of BSG can achieve up to 40% dry matter content while requiring a fraction (<25%) of the energy compared to thermal drying. **(Objective 1.1)**

Screw press dewatered BSG in bench scale stirred reactor and SE HTT

2. **<u>Screw press dewatering is an efficient method for fractionating and improving the biomass for HTT</u>**

   (i) Screw press dewatering, apart from moisture reduction, selectively reduced solubles and proteins from BSG that negatively affect XOS product purity in the hydrolysate. In both the stirred batch reactor and the SE the purity of XOS (XOS+ArOS/TDS) increased with higher level of screw pressing.

      1) The purity of hydrolysate increased with higher level of screw pressing. In the ELA dosed factorials, the components in the hydrolysate not determined (TDS ND), excluding oligomers, sugars and inhibitors or degradation products, decreased from an average 11.4 g/100 g dm, with 15% dm BSG, to 9.5 g/100 g dm from 25% dm BSG.

      2) The highest purity XOS product was obtained in SE HTT, 41.2% and 51.9% of TDS, Weiss and Malt BSG respectively, both from 32% dm.

(ii) The mechanical fractionation of BSG by screw press create valuable by-products. The press liquid fraction removed by dewatering of BSG is a source of valuable vegetable protein. The suspended solids in the liquid removed from Weiss and malt BSG contained 39.5% (43.0% amino acids). The clear liquid fraction is high in soluble minerals that can be used in food applications.

3. **Screw press dewatering is an efficient method for modification of biomass to improve acidification in HTT**

Additionally, a screw press selectively increased the fibre fraction of BSG by reduction of proteins, starch and soluble impurities which increased the purity of the XOS product in the hydrolysate produced after HTT. Moreover the small changes in BSG composition as a result of the selective removal of components by the screw press dewatering can lead to enhanced acidification or autocatalytic H+ production (mol H+ produced per gram BSG dry) in the HTT process by reduction of the buffering capacity in the BSG (Jacobsen and Wyman, 2000; Liao *et al.*, 2004). This increased acidification and reduced moisture content in BSG together increased H+ concentration that proportionally accelerates the solubilisation and depolymerisation of hemicellulose xylan by hydrolysis (Mosier, Ladisch and Ladisch, 2002). Overall, this enhanced/increased H+ concentration effect in HTT as a result of screw press dewatering was substantially different in the HTT systems, stirred batch reactor compared to SE and in autocatalytic operation compared to with ELA dosing:

(i) In autocatalytic (no added catalyst) HTT

a) Stirred batch reactor HTT (Chapter 4)

i. Screw press dewatering can achieve significantly increased CSF in autocatalytic LHW HTT achieved through lowered pH in the hydrolysates from enhanced HTT acidification with increased H+ concentration obtained (approximating by the water reduction). The screw press dewatering of BSG from 15% to 25% dm (*circa* 47% moisture reduction) lead to significantly increased H+ concentration (*circa* 100%) in the autocatalytic LHW HTT at 150 and 180 ºC tested. At both temperatures of 150 ºC and 180 ºC the lowered pH obtained (pH 4.38/4.05 and 3.84/3.54) from 15% compared to 25% dm BSG is proportionally equivalent to the difference in water content of the BSGs. **(Objective 1.5.(i))**

ii. Small compositional differences as a result from screw press dewatering of BSGs are not significant in autocatalytic LHW HTT acidification since raw and screw pressed BSG resulted in comparable acidification results (mol H+ produced per gram BSG dry) for each temperature 150 ºC and 180 ºC. The pH

changes and H+ concentration obtained are equivalent to a constant HTT acidification, mol H+ produced per gram BSG dry, for both 15% and 25% dm BSG in autocatalytic LHW HTT, at temperature 150 ºC (2.49 x 10-7 mol/g) and 180 ºC (8.35 x 10-7 mol/g).

iii. Compared to screw press dewatering, temperature is a more significant factor in the BSG hydrolysate acidification in autocatalytic LHW HTT. Results show a 9% increased temperature (150 ºC to 180 ºC) resulted in a significantly increased (> 230%) [H+] concentration compared to only *ca* 100% increased [H+] concentration obtained from the moisture reduction of 47% between raw to screw pressed BSG.**(Objective 1.5.(i))**

b) Steam explosion HTT (Chapter 5)

Overall, this enhanced/increased H+ concentration effect in HTT as a result of screw press dewatering was substantially different at 180 ºC and 200 ºC in the autocatalytic SE HTT reactor system:

i. Screw press dewatering is effective for enhanced SE HTT of BSG by increased CSF achieved through lowered pH in the hydrolysates and screw press dewatering can obtain an optimum acidification in SE. In autocatalytic SE at 180 ºC and 10 min (while at a much reduced acidification level <20% compared to at 200 ºC) the screw press dewatering of BSG from 15% to 25% dm (*circa* 47% moisture reduction) lead to significantly increased H+ concentration (*ca* 300%). Screw press dewatering can obtain an optimum acidification in SE since a further dewatering to 32% dm lead to a 9% decrease in acidification. **(Objective 1.7.(ii))**

ii. Screw press dewatering is equivalent to moisture reduction by air drying in autocatalytic SE HTT. The increased acidification effect (mol H+ produced per gram BSG dry) in SE HHT at 200 ºC and 5 min from screw press dewatering of BSG from 25% to 32% dm (*ca* 29% moisture reduction) was comparable to the proportional increased acidification in SE HTT as a result of moisture reduction from air dried BSG. **(Objective 1.7.(i))**

iii. SE HTT system without stirring is less suitable for low dry matter concentration (< 25%) BSG HTT compared to the stirred batch reactors. The screw press dewatering of BSG from 15% to 25% dm (*circa* 47% moisture reduction) increased acidification (mol H+ produced per gram BSG dry) in SE HTT at 180 ºC significantly (up to 117%) yet screw press dewatering of BSG showed no significant change in acidification between 15% and 25% dm (*ca* 8.35 x 10-7 mol/g) at 180 ºC in batch stirred reactors. **(Objective 1.7.(ii))**

iv. Small compositional differences as a result from screw press dewatering of BSGs are not significant in autocatalytic SE HTT. Comparing screw pressed BSG's from two different types of BSG in autocatalytic SE HTT found comparable trends in the resulting hydrolysate acidification results **(Objective 1.6.(iii))**

v. Compared to the screw press dewatering, small differences in BSG composition in autocatalytic SE HTT is less significant on the xylan solubilisation and depolymerisation. Results show two different BSG achieved comparable yields from similar screw press dewatered moisture levels. Both types of BSG's obtained the highest XOS yields (>73%) form 25% dm (level A pressed) at 180 ºC and 10 min. **(Objective 1.6.(iii))**

(ii) ELA dosed LHW HTT (Chapter 4)

i. Screw press dewatering can achieve significantly increased CSF in ELA dosed LHW HTT achieved through increased H+ concentration, more than the equivalent of the water reduction such as in autocatalytic LHW HTT. The screw press dewatering of BSG from 15% to 25% dm (ca 47% moisture reduction) lead to significantly increased H+ concentration (up to *circa* 640%) in ELA dosed LHW HTT. **(Objective 1.5.(ii))**

ii. Screw press dewatering does not affect the overall buffering capacity of BSG significantly since the solid pressed phase contains a large part. This increased H+ concentration effect showed a negative relationship with treatment time. At temperatures of 130 ºC in ELA HTT at 5 min (20 mg H_2SO_4/g BSG) the pH obtained lowered (pH 2.40/1.84) from 15% compared to 25% dm BSG, yet at 15 min increase (pH 2.89/1.95) again. **(Objective 1.5.(ii))**

4. <u>Moisture content as additional HTT process variable for optimisation</u>

To conduct an optimisation in HTT of biomass, such as BSG, the effect of the initial moisture content needs to be considered as a variable in addition to processing time and temperature.

(i) <u>Batch stirred reactor HTT system (Chapter 4)</u>

Optimisations for HTT of biomass in stirred batch reactors are generally conducted at fixed and low <11% dm while optimising for treatment time and temperature. Yet initial moisture content affects the [H+] concentration in the HTT process that affect the rate of polysaccharide solubilisation and depolymerisation. This study showed in the autocatalytic (no added catalyst) LHW HTT the [H+] concentration could be significantly varied (up to 100%) by changing the initial moisture content between 85 to 75% wt. (15 to 25% dm). The acidification effect obtained in the autocatalytic LHW HTT at 180 ºC and 5 min treatment (15 to 25% dm) resulted in XOS yields (65.0 to

65.3%) which are comparable to maximums XOS yield (61%) from reported optimisations at 190 ºC and 5 min (11% dm). Additionally, the degradation products obtained in this study (1.11 and 1.49 g/100 g BSG) are significantly lower at the reduced treatment temperatures (by 10 ºC) used compared to that reported (1.89 g/100 g BSG) at optimised process condition. Therefore, the inclusion of moisture content as a variable or independent factor, in addition to temperature and treatment time, allows for LHW HTT optimisations to obtain true maximum XOS yields; even at reduced temperatures and with less degradation by-product formation. **(Objective 1.2.(i))**

(ii) Steam explosion reactor HTT system (Chapter 5)

Similarly, in SE HTT optimisations, an arbitrarily chosen and a fixed moisture content is also used, usually >11% dm. This study showed in the SE HTT of BSG at 200 ºC (optimum process conditions for XOS production from literature) that the [H+] concentration could be significantly varied (up to 4000%) by changing the initial moisture content (between 75 to 10% wt.) that resulted in significantly different product yields and degradation by-products formation. The effect of the initial moisture content on acidification effect in HTT and optimisation for XOS production from BSG was further demonstrated at 180 ºC in SE (preferred process conditions from literature) were 25% dry matter concentration resulted in the highest XOS yield (75.3%) when compared to 15 % dm (21.1%) and 32% dm (48.3%) contents. An increased dry matter concentration from 15% to 25% resulted in increased (>300%) [H+] concentration yet a further increase in dry matter from 25 to 32% resulted in a decrease (9%) in [H+] concentration in the final hydrolysate. Clearly for comprehensive investigations in HTT of biomass the water content must be varied along with other process parameters. **(Objective 1.6.(i))**

5. Increased solids loading (>11% dm) in HTT can lead to significant process advantages:

The use of higher dry matter concentrations (>11% dm) in HTT can have significant process advantages if the negative effects of increased inhibitors formation on process yields can be mitigated.

(i) **Higher XOS product yields in HTT**

Based on literature, it is to be expected that higher dm in HTT will have negative effects on process yields, while increasing inhibitors formation. Yet, contrary to that expected, in both chapter 4 and 5 these disadvantages were overcome by appropriate process optimisation strategies and it was shown in this study that increased initial dry matter content (>15% dm) for HTT in stirred batch reactors (Parr) and SE can lead to

significant (>10%) XOS yields increases at preferred process conditions for autocatalytic HTT of BSG.

a) Stirred batch reactor HTT (Chapter 4)

A XOS yield of 78.0% was obtained in stirred batch reactors at 180 ºC and 15 min using 15% dm compared to reported XOS yields at 11% dm of 61% at 190 ºC and 5 min and 67% at 180 ºC and 12.2 min using similar reactors. **(Objective 1.2.(i))**

b) *Steam explosion HTT (Chapter 5)*

Furthermore, using 25% dm BSG in SE HTT a 75.3% XOS yield was obtained at 180 ºC and 10 min compared to only 21.1% using 15% dm. These results are contrary to literature that says increasing solids loading will have a negative effect on product yields such as XOS, and typically resulting in increased sugars degradation and by-products formation (Modenbach and Nokes, 2012). Yet this improvement can be as a result of the changes effected on BSG by screw press being a combination of physical/buffering (Brownell and Saddler, 1986) and changes in chemical buffering (Jacobsen and Wyman, 2000; Mosier, Ladisch and Ladisch, 2002; Liao *et al.*, 2004) as a result of the dewatering.**(Objective 1.6.(i))**

(ii) **Process intensification in HTT**

a) Stirred batch reactor HTT (Chapter 4)

Increased initial dry matter content (>15% dm) for HTT in stirred batch reactors (Parr) can lead to significant process intensification. This study showed using higher dry matter concentrations (>15%) of BSG in a HTT process can provide:

(i) an increased product concentrations for example XOS concentration increased from 16.6 g/l to 31.7 g/l using 25% dm compared to 15% dm,
(ii) a reduction in the required process water of up to 60%,
(iii) (ii) reduced process temperatures of between 20 to 40 ºC compared to reported 9 to 11% dm LHW HTT of BSG while maintaining or increasing product yields, and
(iv) a reduction in degradation by-product formation as was shown in the application of appropriate high solids HTT processing of BSG in autocatalytic LHW HTT at 15% dm (78.0% XOS yield at 180 ºC and 15 min) that resulted in degradation by-product formation of only 1.50 g/100 g dm compared to 1.89 g/100 g dm reported at optimised stirred batch conducted at 11% dm (61% XOS yield at 190 ºC and 5 min).

b) *Steam explosion HTT Chapter 5)*

This study showed using higher dry matter concentrations (>15%) of BSG in a SE HTT process can provide:

(i) a reduction in the required process water in HTT of more than 60%,

(ii) reduced HTT process temperatures of up to 20 ºC compared to reported 9 to 11% dm LHW HTT of BSG while maintaining or increasing product yields, and

(iii) a further reduction in degradation by-product formation compared to stirred batch work (Chapter 4) as was shown in the application of appropriate high solids HTT processing of BSG in SE at 25% dm (75.3% XOS yield) that resulted in degradation by-product formation of only 0.81 g/100 g dm compared to 1.50 g/100 g dm with 15% dm (Chapter 4 - 78.0% XOS yield) and 1.89 g/100 g dm reported at optimised conditions (190 ºC and 5 min in stirred batch conducted with 11% dm (61% XOS yield).

Thus, the combination of effects of higher XOS concentrations, lower water usage, lower processing temperature requirements and lower degradation by-product formation achieved in this study could lower process energy and reduced equipment requirements that combined contributed to production costs that enabled BSG valorisation at small scale.

6. **Xylose yields at preferred dilute acid (100 mg H_2SO_4/ g dm) LHW HTT conditions are more sensitive to screw press dewatering compared to XOS using no acid**

   The screening result showed XOS yields from optimised process conditions for autocatalytic (no acid added) LHW were comparable for raw BSG (65.0%) and screw pressed BSG (65.3%), yet xylose yields increased significantly from 57.3% for raw BSG to 71.8% for screw pressed BSG. **(Objective 1.2.(ii))**

7. **High (>80%) total xylan recovery can be achieved with near 50/50 xylose/XOS selectivity at process conditions selected in the range between autocatalytic and dilute acid optimum process conditions reported**

   Screening results show while (no acid catalyst added) autocatalytic LHW HTT produced mainly XOS (XOS% 93.0% for 180 ºC 5 min for both 15% dm and 25% dm) an acid dosed (100 mg H_2SO_4/ g dm) LHW HTT produced mainly xylose (XOS% 33.5% and 8.6% at 120 ºC for 15% dm and 25% dm respectively) the 46 mg H_2SO_4/ g dm acid dosing at 120 ºC recovered > 80% BSG xylan in a near equal proportion of XOS and xylose (XOS% 57.8% and 55.8% at 120 ºC for 15% dm and 25% dm respectively) **(Objective 1.3)**

8. Extremely low acid (ELA) dosing for improving autocatalytic HTT technology

c) Stirred batch reactor HTT (Chapter 4)

(i) ELA dosing as low as 5 mg H_2SO_4/ g dm can enhance the autocatalytic LHW HTT at high (>15% dm) solids loading in stirred batch reactors

Results from the factorial experiments of the ELA dosed LHW HTT in the stirred batch reactors for both raw 15% dm and 25% dm screw pressed BSG found the acid loadings applied to be significant factor and in combination with temperature for XOS and xylose production. Maximum XOS yields of 76.4% and 65.5% were found at 170 ºC and 5 mg, for both, after 15 min and 5 min respectively for raw 15% dm BSG and 25% dm screw pressed BSG. **(Objective 1.4.(i))**

(ii) ELA dosing can mitigate the negative effects of high solids (15%dm) loading HTT in stirred batch reactors

The advantage of ELA contributed to counteracting the negative effects from increased dry matter content HTT processing by achieving similar of improved product yields, such as XOS, in HTT of biomass at high (>15% dm) solids loading compared to autocatalytic LHW HTT. This is achieved by:

(i) reduced process temperatures from 180 ºC to 170 ºC. ELA dosed catalyst as low as ~5 mg H_2SO_2/g BSG to LHW HTT (15% dm) resulted in similar XOS product yields (65.0/78.0%) at 170 ºC for 5 and 15 min when compared to yield (67.7/76.4%) from autocatalytic LHW HTT at 180 ºC. **(Objective 1.4.(i))**

(ii) reduced degradation and by-product formation. Such a reduction in HTT processing temperatures results in significantly reduced by-product or degradation products formation as a result of the lower rate of formation from these temperature sensitive reactions (Section 4.3.3.3). Degradation products yields from autocatalytic LHW HTT of BSG (15% dm) at 180 ºC for 5 and 15 min, 0.71 and 1.18 g/ 100 g dm, reduced significantly at 170 ºC to yield 1.11 and 1.50 g/ 100 g dm respectively. This application of ELA dosing to LHW HTT can lead to lower energy usage (lower process temperatures) and generation of wastes (lower degradation by-products) associated with LHW HTT. **(Objective 1.4.(i))**

(iii) ELA dosing in LHW HTT can be aided with CO_2 added at moderate pressure (20 Bar)

(i) The use of CO_2 as a green alternative catalyst can reduce the ELA dosing of corrosive mineral acids in HTT.A mild CO_2 addition to ELA LHW HTT can significantly increase the CSF and reduce the pH further for an enhance HTT process. The addition of 20 Bar CO_2 at 150 ºC and 10 min ELA LHW HTT increased the CSF from -1.29 to 0.15. **(Objective 1.4.(ii))**

(ii) The addition of CO_2 in HTT can be used to depolymerize XOS to increase the xylobiose (X2) and xylotriose (X3) yields. Results from ELA HTT at 150 ºC show the addition of 20 Bar CO_2 (run B-10C), resulted in a near doubling of the X2 and X3 yield in XOS from 7.2% to 15.6%.

a) Steam explosion HTT (Chapter 5)

(i) ELA dosing of SO_2 in SE HTT

In SE solid $K_2S_2O_5$ can be used as alternative SO_2 dosing agent for ELA HTT. Solid $K_2S_2O_5$ addition (30 mg SO_2 eq) in SE HTT of BSG resulted in 56.0 % XOS yield comparable to a liquid SO_2 solution (25 mg SO_2) of 57.7% XOS yield. However, the solid $K_2S_2O_5$ proved milder catalyst since less XOS depolymerisation (XOS% 85.1%) and higher ArOS yield (ArOS yield 27.4%) were found compared that obtained (71.2% and 22.4% respectively) from the liquid SO_2 solution **(Objective 1.6.(iv))**

9. Small variance in BSG feedstock compositions does not affect HTT process significantly

(i) Small variance in feedstock compositions did not affect significantly near optimal process conditions as found in HTT work from batch stirred reactor in the Parr and SE. Two different types of BSG, from a Weiss and Malt brew, resulted in comparable XOS yields (75.1 and 73.1%) in SE for the preferred HTT process conditions at 180 ºC obtained from literature.

(ii) Small compositional differences as a result from screw press dewatering of BSGs are not significant in autocatalytic LHW HTT acidification since raw and screw pressed BSG resulted in comparable acidification results (mol H+ produced per gram BSG dry) for each temperature 150 ºC and 180 ºC. (Conclusion 3.i.a.(ii))

(iii) Small compositional differences as a result from screw press dewatering of BSGs are not significant in autocatalytic SE HTT. For both types of BSG's, screw pressing resulted in similar acidification trends (mol H+ produced per dry gram BSG in the hydrolysate) in SE. (Conclusion 3.i.b.(iv))

10. Process conditions for HTT scale-up from bench scale HTT optimisations

(i) In HTT, the process condition from multivariate optimisations for XOS production from BSG conducted in bench scale batch stirred reactors can be used as near-optimal process conditions for larger SE HTT. Results (this study) showed XOS yield (78.0%) from BSG in autocatalytic LHW HTT in the Parr at 180 ºC and 15 min (15% dm) was within near optimal yield for XOS obtained (75.1%) from SE at 180 ºC 15 min using the same BSG (25% dm). This was in agreement with literature reports that showed near optimal HTT process conditions of time and temperature identified through multi-variate optimisations for total xylan yield from

biomass conducted in the smaller, bench scale (10 g) reactors under LHW conditions could be scaled-up to the pilot scale (>500 kg/d) reactor systems including SE reactors (Lischeske *et al.*, 2016). The near-optimal yields space (two standard deviations from the optimum) from larger systems, cannot be distinguished from the optimal result in the smaller bench reactor with a confidence of 95%.

(ii) Dry matter content was shown to be an important process variable that enable SE HTT to achieve the comparable yields. This can be as a result of the improved rate of heat transfer obtained from steam with dewatering BSG in the unstirred SE. Measured acidification (mol H+/g dry BSG) obtained from HTT at 180 ºC in the stirred Parr reactor remained fairly constant for different levels of screw press dewatering (Conclusion 3.(i)) while in SE it had a significant impact.(Conclusion 4.(ii)).

(iii) Cost efficient method for HTT scale-up by using optimal process conditions from bench scale multivariate optimisations or even literature reported data: Bench scale optimal process conditions reported for XOS production was shown to scale-up in larger HTT systems.

(iv) HTT results/yields can be matched between HTT systems. Hemicellulose solubilisation optimisation results from HTT in a bench Parr reactor can be expected to be replicated in other equipment even at pilot scale, such as in a steam gun. The main biomass solubilisation and depolymerisation chemistry should remain more or less the same for the various different processing equipment or from different heating applied. Initial moisture content in BSG was shown to be a significant process factor that can be adjusted to achieve comparable HTT results, to compensate to an extent for the limitations in mass and heat transfer behaviour changes between various reactor types.

11. <u>CSF is a useful measure for performance comparison between HTT systems</u>

(i) Using CSF results of XOS and total xylan yield from LHW and ELA HTT in stirred batch rectors overlapped with continuous screw reactor from literature. **(Section 4.3.7)**

(ii) XOS and total xylan equivalent yields for reported autocatalytic LHW HTT and SE HTT in this study overlap when compared with CSF. **(Section 5.3.2.4)**

B. Objective #2: Techno-economic assessment of novel processes for the valorisation of BSG

12. Profitable small scale biorefinery annexed to a large brewery producing high value products from BSG using high solids HTT technology enabling

Using the results from the HTT work in a techno-economic assessment of a valorisation case for BSG provided economic benefits that made all three small scale biorefinery concepts producing XOS, xylitol and both together, profitable with an IRR larger than the hurdle rate (9.7%) when considering a conservative market price for xylitol and XOS as US$4500/t.

(i) The scenario for XOS production was more profitable than xylitol with XOS minimum required selling price (MRSP) of US$2509/t compared to US$4153/t for xylitol. The high xylan equivalent (XOS+xylose) yield obtained (~80%) at the highest XOS yield process condition (180 ºC 10 min) in SE HTT from BSG at 25 % dry matter concentration, significantly improved the overall production of both XOS and xylitol, compared to optimised process in literature (<70% XOS+xylose at 190 ºC 5 min 9-11% dm) that used lower 11 % dry matter concentration. However, the complexity of CAPEX and OPEX and cash-flow, maximum yield of the XOS did not result in the best economic performance overall. **(Objective 2.)**

(ii) The scenario producing both XOS and xylitol together achieved the lowest MRSP of US$2182/t. Using the same dry matter (25 % dm), the 46 mg ELA H_2SO_4 dosed LHW HTT used reduced temperature (120 ºC) and achieved higher xylan equivalent yield (~85%) compared to SE HTT to contribute to the overall higher profitability of the scenario that produced both XOS and xylitol.

(iii) BDG protein (BDP) and feedstock cost were the two factors with the biggest effect on minimum selling prices of the products. The revenue from the selling of BDP is an important part and a decrease in the value from US$350/t to US$100/t increased the MRSP for xylitol to US$5132/t from US$4153/t. The BSG cost had the largest effect on MRSP of the xylitol and XOS. Lowering the cost of BSG feedstock to US$0/t had the largest decrease of MRSP in all scenarios with scenario C reaching the lowest US$1517/t for xylitol and XOS and to US$2939/t for xylitol alone in scenario A. A near zero BSG feedstock cost is not impossible for a small scale biorefinery adjacent to a brewery since many breweries do not have the option to sell their BSG to farmers.

7.2 Recommendations

1 **Measure buffering capacity in raw and screw pressed BSG**

Determining the buffering capacity of the various BSG's and the contribution of each the liquid and solid fractions will go a long way to help explaining their possible contribution of each in the effects seen in the HTT experiments.

2 **Development of a buffering capacity test for biomass for quick HTT system evaluations**

Optimisations of hydrothermal treatments need to consider buffering capacity of the biomass treated, especially in autocatalytic and ELA HTT. Although compositional acetyl groups can give an indication of the acidification potential the buffering capacity can be significant as was demonstrated with ELA HTT experiments.

3 **Further investigation of the use of CO_2 in autocatalytic HTT systems**

The inclusion of CO_2 in ELA HTT was demonstrated at moderate pressures. CO_2 can be used to reduce the use of mineral acids when applied to enhance the autocatalytic HTT at lower process temperatures. The possible interaction of acid and CO_2 must be investigated since it is environmentally friendly and can reduce the use of mineral acids.

4 **Re-dilution tests with screw pressed BSG for maximum effect from screw pressing**

Screw press dewatering tests can be further extended to use a maximum dewatering *circa* 40% dry matter BSG while diluting with water to obtain optimal hemicellulose solubilisation and depolymerisation products XOS and xylose.

5 **Do more economic models and quantitative environmental impact assessment**

Such studies will demonstrate more clearly what the economic and environmental benefits of high solids are.

6 **Scale-up to continuous HTT, industrial systems**

Continuous processing of lignocellulosic materials is more widely applied industrially and such continuous processing data from BSG could further improve the accuaracy of the techno-economic analysis.

Appendix: Publications

Provisional Patent P3471ZA00/SC

AS FILED

SOUTH AFRICAN COMPLETE PATENT APPLICATION

Title: PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF

Patent Application No: 2019/00975

Date of Filing: 15 February 2019

Priority Claimed: South Africa Patent Application No. 2017/07724
Dated 15 November 2017

Applicant(s): STELLENBOSCH UNIVERSITY

Inventor(s): SWART, Lukas Johannes, GÖRGENS, Johann Ferdinand, VAN RENSBURG, Eugene

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FORM P1

REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT

[Section 30 (1)-Regulation 22]

The granting of a patent is hereby requested by the undermentioned applicant on the basis of the present application.

Official Application No.			Applicant's or Agent's Reference
21	01	**2019/00975**	P3471ZA01SC

71	Full Name(s) of Applicant(s)

STELLENBOSCH UNIVERSITY
Admin B, Victoria Street,, Stellenbosch, Western Cape Province, 7600, South Africa

54	Title of invention

PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF

The applicant claims priority as set out on the accompanying Form P.2. The earliest priority claimed is
COUNTRY: ZA NUMBER: 2017/07724 DATE: 2017/11/15

~~This application is for a patent of addition to patent application No.~~

21	01	

~~This application is a fresh application in terms of section 37 and based on Application No.~~

21	01	

This application is accompanied by:

X	1.	A single copy of a complete specification of 10 pages.
X	2.	Drawings of 5 sheet(s).
X	3.	Publication particulars and Abstract(Form P8)
	4.	A copy of a figure of the drawing (if any) for the abstract
	5.	Assignment of invention
X	6.	Certified priority document(s)
	7.	Translation(s) of the priority document(s)
	8.	Assignment of priority rights
X	9.	A copy of the Form P.2 and the specification of S.A Patent Application (if applicable).
	10.	A declaration and power of attorney on Form P3
	11.	Statement on the use of indigenous Biological Resource, Genetic Resource, Traditional Knowledge or Use on Form P26
X	12.	Form P4 - Other requests not specified
X	13.	Other Supporting Document

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SOUTH AFRICA

Dated this 15th day of February 2019

Submitted online by : Von Seidels

.....
Signature of Applicant(s)

This is returned to the applicant's
address for service as proof of lodging.

RECEIVED

Official Date Stamp

.....
Registrar of Patents

FORM P2

REPUBLIC OF SOUTH AFRICA | REGISTER OF PATENTS | PATENTS ACT, 1978

Official application No.	Lodging date: Provisional	Acceptance date
21 01 **2019/00975**	22	47
International classification	Lodging date: Complete	Granted date
51 A23L;C12C;C12F;C12P	23 2019/02/15	

71 Full name(s) of applicant(s)/Patentee(s):

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71 Applicant substitued:	Date registrered
71 Assignee(s):	Date registrered

72 Full name(s) of inventor(s):

STELLENBOSCH UNIVERSITY

Priority claimed:	Country	Number	Date
	33 ZA	31 2017/07724	32 2017/11/15

54 Title of invention

PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF

Address of applicant(s)/patentee(s):

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SOUTH AFRICA
Reference No. P3471ZA01SC

61 Patent of addition No.	Date of any change
Fresh application based on.	Date of any change

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978
APPLICATION OR REQUEST TO THE REGISTRAR
(REGULATION 39)

OFFICIAL APPLICATION NO.		
21	01	**2019/00975**

AGENT REFERENCE
P3471ZA01SC

IN THE NAME OF:	
71	STELLENBOSCH UNIVERSITY

In terms of the following section(s) Section 31 (1) of the Act and/or regulation(s) 23-25, 39 of the Patent Regulations, the applicant hereby request the following:

a three month extension of time for claiming priority

Documents, if any, lodged in support of the request:
N/A

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15 February 2019

Submitted online by : Von Seidels

Signature of Applicant

FOR OFFICIAL USE ONLY

The above application or request is hereby allowed/refused.

Reasons for refusal of conditions of allowance, if any:

OFFICIAL DATE STAMP

REGISTRAR OF PATENTS

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978
COMPLETE SPECIFICATION
[Section 30(1) – Regulation 28]

OFFICIAL APPLICATION NO.		LODGING DATE	
21 01	**2019/00975**	22	2019/02/15

INTERNATIONAL CLASSIFICATION	
51	A23L;C12C;C12F;C12P

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FULL NAME(S) OF INVENTORS(S)

72	1. STELLENBOSCH UNIVERSITY

TITLE OF INVENTION

54	**PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF**

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

PUBLICATION PARTICULARS AND ABSTRACT
[Section 32(3)(a) – Regulation 2291)(g) AND 31]

OFFICIAL APPLICATION NO.		LODGING DATE		ACCEPTANCE DATE	
21	01 **2019/00975**	22	2019/02/15	47	

INTERNATIONAL CLASSIFICATION		NOT FOR PUBLICATION
51	A23L;C12C;C12F;C12P	CLASSIFIED BY: Von Seidels

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FULL NAME(S) OF INVENTORS(S)

72	1. STELLENBOSCH UNIVERSITY

EARLIEST PRIORITY CLAIMED

COUNTRY		NUMBER		DATE	
33	ZA	31	2017/07724	32	2017/11/15

TITLE OF INVENTION

54	**PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF**

57	**A process for producing an extract from brewers' spent grain (BSG) is provided. In the process, BSG is hydrolysed and a liquid fraction of the hydrolysed BSG is then recovered as the extract. The extract has a reduced starch, protein, lipid, fat and wax content relative to unhydrolysed BSG. A preconditioning step may be performed on the BSG before hydrolysis, e.g. so as to reduce its water content. The hydrolysed BSG may also be subjected to one or more post-hydrolysis conditioning steps, such as a separation step to remove solids, pH adjustment, concentration, filtration, activated carbon adsorption, colour adjustment, ion exchange, solvent extraction, acid catalysis and/or conditioning with a microorganism or enzyme. The extract can be added to a food product or to a process for making a food product. A food product containing the extract, such as a beer or animal feed, is also provided.**

PROCESS FOR PRODUCING A BREWERS' SPENT GRAIN EXTRACT AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from South African provisional patent application number 2017/07724 filed on 15 November 2017.

FIELD OF THE INVENTION

The invention relates to a process for producing an extract from brewers' spent grain and also to uses of the extract, especially in producing a food product which has been fortified with the extract.

BACKGROUND TO THE INVENTION

Beer is made by saccharification and fermentation of a starchy plant material, such as wheat, rye, oats, malted barley, corn, sorghum, rice, millet, sugar, cassava, potato and agave, or a combination of these. During the brewing process, the starchy material is subjected to enzymatic degradation, resulting in the liberation of fermentable carbohydrates (maltose and maltotriose), non-fermentable carbohydrates (dextrins), soluble proteins, polypeptides and amino acids. This process, referred to as "mashing", results in a mash which comprises a soluble (liquid) medium known as wort and insoluble components known as "brewers' spent grain" (BSG). The wort is separated from the BSG and is fermented into beer by the action of yeast.

BSG is by far the largest by-product of the brewing industry, with about 40 million tonnes of BSG being produced annually. A portion of the BSG is used in the manufacture of low-value animal feeds, but disposing of the remainder of this by-product is both an environmental and financial concern. There is therefore a need for alternative uses for BSG.

SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided a process for producing an extract of brewers' spent grain (BSG), the process comprising the steps of:

hydrolysing BSG; and

recovering a liquid fraction of the hydrolysed BSG.

The liquid fraction of the hydrolysed BSG may have a reduced starch, protein, lipid, fat and wax content relative to the unhydrolysed BSG.

The BSG may be hydrolysed by way of steam explosion.

A preconditioning step may be performed on the BSG prior to hydrolysis. For example, the BSG may be subjected to a pressing step so as to reduce the water content of the BSG.

The process may also comprise one or more post-hydrolysis steps being performed on the hydrolysed BSG. These steps may include one or more of the following: a separation step to remove solids from the hydrolysed BSG, pH adjustment, concentration, filtration, activated carbon adsorption, colour adjustment, ion exchange, solvent extraction, acid catalysis and/or conditioning with a microorganism or enzyme.

According to a second embodiment of the invention, there is provided a BSG extract produced by the process described above.

According to a third embodiment of the invention, there is provided a process for producing a food product, the process comprising the step of adding a BSG extract described above to a food product or to a composition which is used to manufacture the food product.

The food product may be an edible product or beverage for human consumption, such as a beer or beer product. Alternatively, the food product may be an animal feed.

According to a fourth embodiment of the invention, there is provided a food product which includes a BSG extract as described above. The food product may be an edible product or beverage for human consumption, such as a beer or beer product. Alternatively, the food product may be an animal feed.

DETAILED DESCRIPTION OF THE INVENTION

A process for producing an extract from brewers' spent grain (BSG), the use of this extract for producing a food product and a food product containing the extract are described herein.

The term "brewers' spent grain" (BSG) as used herein refers to a secondary product from wort production, in particular the insoluble components of the mash after lautering. The BSG can be from wheat, rye, oats, malted barley, corn, sorghum, rice, millet, sugar, cassava, potato or agave. "Raw" BSG is BSG which has not been subjected to any preconditioning steps prior to the hydrolysis step described in the invention.

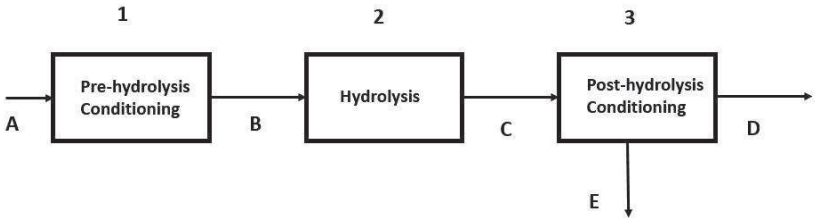
The term "food product" as used herein refers to any edible food product and includes liquid beverages.

The term "beer" or "beer product" as used herein refers to a fermented beverage produced from wheat, rye, oats, malted barley, corn, sorghum, rice, millet, sugar, cassava, potato or agave, or a combination thereof. The beer may be an alcoholic or non-alcoholic beverage.

Throughout the specification and claims, unless the contents requires otherwise, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

In the process of the present invention, BSG is hydrolysed and a liquid fraction of the hydrolysed BSG is recovered as the BSG extract. Optionally, the water content of raw BSG can be reduced prior to hydrolysis, and one or more post-hydrolysis conditioning steps can also be performed to concentrate, purify and/or modify the liquid fraction of the hydrolysed BSG.

An embodiment of the process for producing the BSG extract is summarised in a three-step block flow diagram, shown in scheme 1 (where steps 1 and 3 are optional).



Scheme 1: Generalised block flow for BSG processing

Step 1 (optional)

In step 1, raw BSG (stream A) from a brewing process is subjected to a pre-hydrolysis conditioning step to decrease its water content and to remove fines. The raw BSG typically has a dry mass (dm)% (w/w) varying anywhere from 5-25%. In one embodiment, the pre-hydrolysis step can be performed by way of a mechanical conditioning step, such as by using a screw press. Other mechanical conditioning steps include milling by hammer mill, roller mill or disk mill, or pressing by flat press or filter press. This step can also be performed by washing, diluting with water or filtering (e.g. using a screen or sieve); by drying or dewatering (e.g. with steam/heat and/or drying by air); by chemical conditioning (e.g. by addition of an acid or alkali, alone or in conjunction with the addition of a gas such as SO_2 or CO_2 or with a solvent); by processing (e.g. for starch removal); by enzymatic treatment (e.g. with a protease, cellulase or amylase), or any combination thereof.

Typically, about 40% (w/w) and preferably 50% (w/w) or more of the water, starches, proteins, lipids, fats, waxes and other water soluble compounds in the BSG are removed in this step (Table 1). The essentially solid BSG which remains after this step thus has a reduced concentration of water, starches, proteins, lipids, fats and waxes compared to the raw BSG. On the other hand, the concentrations of components of the BSG which are not removed in this pre-treatment step (e.g. ash, hemicellulose, cellulose and lignin) are increased.

Table 1: Exemplary reduction of components in the pre-treatment step

Components on dry basis	General BSG compositions	Preconditioned BSG	
Water	70-85%	60-75%	50% or more reduction
Starch	2-20%	<5%	Reduced under 5%
Protein	10-28%	5-15%	50% or more reduction
Lipds, Fats & Waxes	6-16%	4-10%	40% or more reduction
Water Solubles	6-30%	3-15%	50% or more reduction

Step 2

Either raw BSG or BSG which has been pre-conditioned as described above is hydrolysed, for example by way of steam explosion. Steam explosion is a well-known process in which biomass is treated with hot steam (110 to 240°C) under pressure (1 to 3.5MPa), followed by an explosive decompression of the biomass that results in a rupture of the biomass fibres. The BSG material thus opens up, without requiring chemical treatment or a mechanical step such as grinding. In the present invention, the steam explosion of BSG is typically performed under low severity conditions, i.e. at a temperature of from about 170-200°C for a period of from about 5-15 minutes and under a pressure of from about 1.5-2MPa. Optionally, an additional catalyst can be added during hydrolysis, such as an acid (e.g. H_2SO_4, H_3PO_4, HCl, formic acid or alkali KOH), NaOH, $Ca(OH)_2$, a salt (e.g. $K_2S_2O_7$, $Na_2S_2O_7$ or $Mg(HSO_3)_2$) or a gas (e.g. SO_2 or CO_2), or a combination thereof. The steam explosion process can be run in a continuous or batch mode. In a continuous hydrolysis reactor, a counter-current solid to liquid flow is particularly suitable. Instead of steam explosion, other methods of causing the BSG material to open up may be used, e.g. steam refining or processes which do not create an explosion.

Step 3 (optional)

In some hydrolysis processes (for example in continuous counter-current flow reactors), liquid and solid products are separated in the hydrolysis process, and stream C will be a hydrolysis syrup. However, after other hydrolysis processes, stream C may be a slurry product and a solids liquid separation step is required to separate stream C into a liquids (or syrup) fraction (stream D) and a solids fraction (stream E). This can be performed by centrifuging, filtering, settling or decanting, or if stream C has a high solids to liquid ratio, by pressing, screw-pressing or filter-pressing.

Post-hydrolysis conditioning steps to concentrate, purify and/or modify stream D (or stream C if it is a syrup) can optionally also be performed. These can involve pH adjustment, concentration, filtration (including micro-, nano- or diafiltration), activated carbon adsorption, colour adjustment, ion exchange, solvent extraction, and/or conditioning with a microorganism or enzyme. Saccharides in the syrup can also be depolymerised with an acid catalyst (such as H_2SO_4, H_3PO_4 or HCl), SO_2 or CO_2, or combinations thereof.

Stream D is substantially reduced in starch, protein, hemicellulose, ash and oils content (Table 2) and rich in phenolics and arabinoxylan-oligosaccharides (AXOS) relative to raw BSG. The

sugars and oligomeric sugars contents of the liquid fraction also differ substantially from those of raw BSG. For example, Table 3 shows the sugars content of some different BSG extracts after steam explosion. The arabinoxylan-oligosaccharides (AXOS):xylooligosaccharides (XOS) ratio is important, as the AXOS are the more prebiotic soluble dietary fibres and they are antioxidants. Increasing the arabinoxylan content with an extract from wheat has also previously been claimed to improve the taste and mouthfeel of beer[16]. Phenolics from the BSG, which are also antioxidants, are additionally extracted into the liquid fraction and the applicant has found these to vary in concentration from 150-515mg/l, depending on the source material of the BSG and the process conditions. Table 4 shows some phenolics that have been measured in extracts prepared according to the invention.

Table 2: Components extracted from BSG after steam explosion

Components on dry basis	Components removed in extract
Starch	>90%
Protein	10-40%
Hemicellulose	60-95%
Cellulose	<5%
Ash	>50%

Table 3: Sugars and oligomeric sugars in BSG extracts after steam treatment

	Glc=OS	Xyl-OS	Ara-OS	Glucose	Xylose	Arabinose	AXOS/Xyl-OS
Weis BSG 180ºC 10 min 25%dm	38%	41%	9%	1%	4%	8%	**>6%**
Malt BSG 180ºC 10 min 25%dm	20%	54%	12%	0%	4%	10%	**>5%**
Malt BSG 180ºC 10 min 33%dm	21%	51%	10%	1%	5%	12%	**>7%**
Malt BSG 200ºC 5 min 25%dm	21%	44%	6%	2%	18%	9%	**>27%**

Table 4: Phenolics content in BSG extracts after steam explosion

Phenolic Compound	Malt BSG 180ºC 10 min 25%dm	Malt BSG 200ºC 5 min 25% dm
3,4-Dihydroxybenzoic acid	3%	3%
Vanillic acid	8%	9%
Syringic acid	50%	46%
p-Coumaric acid	14%	18%
Syringaldehyde	3%	5%
Ferulic acid	22%	12%
Coniferaldehyde	0%	7%

Any remaining unhydrolysed BSG solid product is recovered in stream E. This can be used for other purposes (e.g. as an animal feed) or may be disposed of.

Unlike known methods for processing BSG, the process of the present invention does not require grinding of the raw BSG and doesn't require the BSG to be treated with chemicals or enzymes. Additionally, the BSG extract which is utilised (stream D) is a liquids or syrup fraction and not the solids fraction.

The BSG extract can be added to a beer wort, beer or beer product.

When beer wort is filtered and separated from BSG during a typical brewing process, wort compounds such as lipids, nitrogen/MRPs and phenols remain in the BSG fraction[6-9]. This can result in about 30-80% (w/w) of the phenolic compounds being lost from the wort, depending on the filtration technology[5]. This may negatively impact the resulting beer, as phenolics are anti-oxidant compounds that contribute to the reducing power, stability and mouthfeel of beer[10-13]. Like wine, anti-oxidants in beer are reported to have health benefits[17]. For example, moderate consumption of alcoholic beer has been related to lower mortality risks[18-19]. A non-alcoholic contribution to this reduction in mortality risk can be attributed to the variety of natural phytochemicals and nutraceuticals found in beer[19-20]. Some reported health promoting activities are related to dietary fibres[19-21], prebiotics[20-22], anti-oxidants[23-28], anti-carcinogenics[29-31], peptides[31-33], vitamins[19] and minerals[19,34-35]. The removal of phenolics from beer wort during the filtration and separation process can therefore result in a beer with reduced stability and less mouthfeel, and which also has fewer nutrients, nutraceuticals, bioactives or potential health benefits.

Thus, addition of the BSG extract can significantly increase the content of nutraceuticals and other compounds which have potential health benefits in the beer. For example, Table 5 shows a comparison of a few compounds in a typical lager beer compared to a beer to which a BSG extract prepared according to the invention has been prepared. The values of soluble fibres and phenolic acids in the beer with the BSG extract are higher than any reported values for a typical beer.

Table 5: Comparison of compounds in a "normal" beer (without added extract) and a beer with added extract

	Typical Lager beer	50ml raw syrup/l Lager beer
Soluble AX	~0.5 g/l	~3 g/l
Ferrulic Acid	~0.5 mg/l	>2 mg/l
Phenolic Acids	~22 mg/l	>50 mg/l
Colour	~3-4 ºEBC	~20-30 ºEBC

The BSG extract can also be used to adjust the colour of a beer.

The BSG extract can be added during the beer production process or prior to bottling. Typically, about 30-50 ml extract/l beer will be added to the wort, beer or beer product, depending on the content of the liquids fraction and the desired properties of the beer (e.g. taste, mouthfeel, colour).

Importantly, addition of the BSG extract does not negatively affect the taste, colour, mouthfeel, flavours, odour, stability, body and other sensory attributes of the beer. Thus, additional flavour improvers do not need to be added to the beer.

There is no need to use chemicals or additives in the process of the invention, and as the BSG originates from the beer-making process itself, adding the extract to the beer does not result in the introduction of any foreign compounds or material which is not originally from the beer. Instead, the use of the BSG extract is essentially the re-introduction of material which had previously been removed from the beer mash.

In addition to beer, the extract produced by the process described herein can be used as an animal feed or as an additive to an animal feed. Also, as the main constituents of BSG include components which have nutritional value or health benefits to humans (e.g. carbohydrates, fibre, protein, peptides, minerals, vitamins, prebiotics, arabinoxylans and phenolic compounds), the BSG extract can be used as an additive to other human foods or beverages.

The invention will now be described in more detail by way of the following non-limiting examples.

Examples

Example 1

Raw BSG with ~2% residual starch on a dry mass (dm) basis was conditioned with a 2.2kW screw press to remove >50% of the water, thereby also reducing the starch content and soluble dry mass to <1 dm% (w/w). A batch of 120.7kg conditioned BSG was hydrolysed with 15 Barg steam in a 19l batch steam gun with operating conditions of 180ºC and 10 min residence time. The hydrolysis process hydrolysed ~35% of the conditioned BSG. The resulting slurry consisted of ~20% dm% (w/w) and after centrifugation, 83.9kg of syrup was retained. A typical syrup without conditioning contained ~95g/l dissolved solids, 32.7g/l dietary fibre, >2g/l arabinoxylan-oligosaccharides (AXOS), ~1g/l amino acids and phenolics, of which ferulic, coumaric and syringic acid constituted nearly 30%. It was found that a typical dosage of 30ml syrup per litre of beer can double the soluble arabinoxylans and free phenolics. Post conditioning treatment of the syrup, for example using 0.2-3% (w/v) dilute phosphoric acid for 15min at 120ºC, resulted in an increase in soluble arabinoxylans with DP<7 to >50%, and as a result also reduced all the soluble starch to glucose.

Example 2

Similarly to example 1, raw BSG was conditioned with a screw press to reach ~33% dm of conditioned BSG. The conditioned BSG was hydrolysed with 20 Barg steam in a 19l batch steam gun with operating conditions of 200ºC and 5 min residence time. The hydrolysis liquid contained ~35% of the starting dry mass with a concentration of ~124g/l dissolved solids, 30.2g/l dietary fibre and >10g/l AXOS.

Example 3

Similarly to example 1, conditioned raw BSG was processed with 35mg SO_2/g dm in a 19l batch steam gun with operating conditions of 180ºC and 10 min. The process hydrolysed ~41% of the conditioned BSG. The resulting unconditioned syrup contained >25% of soluble arabinoxylans with DP<7. Importantly, the phenolics concentration (ferulic, coumaric and syringic acid) increased 4.5x, 7.4x and 4.6x compared to example 1. These phenolic acids resulted in >50% in total of important phenolics measured.

Example 4

Raw BSG was conditioned by air drying to 94% dm. Conditioned BSG was brought to the required dm% with water and processed with steam as for example 1. The process hydrolysed

~42.4% of the conditioned BSG. A syrup with carbohydrates, soluble arabinoxylans and sugars was obtained as per Table 6.

Table 6: Composition of syrup extract obtained with air drying

Compound	g/l
Gluco-Oligosaccharides	33.4
Arabinoxylo Oligosaccharides	47.6
Glucose	0.7
Xylose	3.3
Arabinose	7.4

Example 5

A raw BSG was conditioned in a flat press to ~40% dm. The conditioned BSG was processed with steam as for example 1. The carbohydrates, soluble arabinoxylans and sugars content of the resultant syrup extract is shown in Table 7.

Table 7: Composition of syrup extract obtained with flat press conditioning

Compound	g/l
Gluco-Oligosaccharides	88.5
Arabinoxylo Oligosaccharides	42.4
Glucose	5.3
Xylose	2.8
Arabinose	5.4

Example 6

In a similar operation as for example 1, screw-pressed conditioned BSG was steam hydrolysed with $K_2S_2O_7$ catalyst at loading, equivalent of 30mg SO_2/g dm loaded and with the hydrolysis processing conditions of 180ºC and 10 min. The resulting unconditioned syrup contained ~140.2g/l of dissolved solids with a carbohydrates, soluble arabinoxylans and sugars content as shown in Table 8.

Table 8: Composition of syrup extract obtained with $K_2S_2O_7$ catalyst

Compound	g/l
Gluco-Oligosaccharides	25.3
Arabinoxylo Oligosaccharides	33.5
Glucose	2.1
Xylose	5.3
Arabinose	8.3

Example 7

Similarly to example 1, raw BSG was first conditioned with a screw press and subsequently air dried to ~90% dm. The conditioned BSG was processed with steam at 200ºC for 5 min. The resulting product had a 59% dm content. The resulting unconditioned syrup had a carbohydrates, soluble arabinoxylans and sugars content as shown in Table 9.

Table 9: Composition of syrup extract obtained with screw press and air drying

Compound	g/l
Gluco-Oligosaccharides	91.5
Arabinoxylo Oligosaccharides	34.2
Glucose	6.1
Xylose	10.3
Arabinose	1.8

Example 8

Raw BSG was conditioned with a screw press to remove >60% of water, leaving a conditioned BSG with ~25% dm. Conditioned BSG was hydrolysed in a horizontal top loading stirred digester with a loaded to reactor surface ratio to operating volume of 0.67. Hydrolysis was performed by external electrical heating at 150ºC for 10 min with added catalyst of 12.5mg H_2SO_4/g dm. About 31% of the conditioned BSG was hydrolysed. The resulting hydrolysis liquid syrup contained >2.5g/l AXOS and ~104g/l dissolved solids and phenolics, with >140mg/l syringic acid concentration.

Example 9

Similarly to example 8, raw BSG was hydrolysed in a horizontal top loading stirred digester with a loaded reactor surface area to operating volume of 0.75. The raw BSG was hydrolysed at process conditions of 130ºC for 15 min with 48mg H_2SO_4/g dm catalyst. The resulting syrup contained carbohydrates, soluble arabinoxylans and sugars with a total of ~83g/l dissolved solids loading that resulted in >50% of soluble arabinoxylans with DP<7.

Example 10

Similarly to example 8, raw BSG was conditioned with a screw press to remove >60% of water, leaving a conditioned BSG with ~25% dm. Conditioned BSG was hydrolysed in a horizontal top loading stirred digester with a loaded to reactor surface ratio to operating volume of 0.56. Hydrolysis was performed by external electrical heating at 150ºC with added catalyst of 12.5mg H_2SO_4/g dm and 20 Bar CO_2 for 10 min. At these conditions, ~35% of the conditioned BSG was hydrolysed, with the resulting hydrolysis slurry containing ~129g/l dissolved solids

and >20% of soluble arabinoxylans with DP<7. A syrup with carbohydrates, soluble arabinoxylans and sugars was obtained as shown in Table 10.

Table 10: Composition of syrup extract obtained after hydrolysis of preconditioned BSG

Compound	g/l
Gluco-Oligosaccharides	24.7
Arabinoxylo Oligosaccharides	25.8
Glucose	2.8
Xylose	8.6
Arabinose	13.2

Example 11

Raw BSG was hydrolysed in a horizontal top loading stirred digester with a loaded operating volume to reactor surface ratio of 0.56. Hydrolysis of raw BSG was at 150ºC for 10 min with 12.5mg H_2SO_4/g dm. At these process conditions, ~41% of the raw BSG dry mass was dissolved in the liquid syrup. Glucose, xylose and arabinose combined comprised <10% of dissolved solids. A syrup with a carbohydrates, soluble arabinoxylans and sugars content as per Table 11 was obtained.

Table 11: Composition of syrup extract obtained after hydrolysis of raw BSG

Compound	g/l
Gluco-Oligosaccharides	28.2
Arabinoxylo Oligosaccharides	17.6
Glucose	0.7
Xylose	1.2
Arabinose	6.3

Example 12

The product syrup from the hydrolysis of raw BSG and screw press conditioned BSG to ~25% dm were compared. Both were hydrolysed in a horizontal top loading stirred digester, heated externally and with a loaded operating volume to reactor surface ratio of 0.56. The hydrolysis processes for both raw BSG and conditioned BSG were performed without catalyst at 180ºC, with residence times of 15 min and 5 min, respectively. Syrup A from the raw BSG and syrup B from the conditioned BSG were obtained with carbohydrates, soluble arabinoxylans and sugars contents as per Table 12.

Table 12: Comparison of syrup extracts obtained from raw BSG and screw press conditioned BSG

Compound	A g/l	B g/l
Gluco-Oligosaccharides	29.4	32.6
Arabinoxylo Oligosaccharides	20.3	38.2
Glucose	1.0	0.8
Xylose	2.2	2.7
Arabinose	3.4	6.1

CLAIMS:

1. A process for producing an extract of brewers' spent grain (BSG), the process comprising the steps of:
   - hydrolysing BSG; and
   - recovering a liquid fraction of the hydrolysed BSG.

2. A process according to claim 1, wherein the liquid fraction of the hydrolysed BSG has a reduced starch, protein, lipid, fat and wax content relative to the unhydrolysed BSG.

3. A process according to either of claims 1 or 2, wherein the BSG is hydrolysed by way of steam explosion.

4. A process according to any one of claims 1 to 3, which further comprises the step of removing water from the BSG prior to hydrolysis.

5. A process according to any one of claims 1 to 4, which further comprises performing one or more post-hydrolysis conditioning steps on the hydrolysed BSG.

6. A process according to claim 5, wherein the post-hydrolysis conditioning steps are selected from: a separation step to remove solids from the hydrolysed BSG, pH adjustment, concentration, filtration, activated carbon adsorption, colour adjustment, ion exchange, solvent extraction, acid catalysis and/or conditioning with a microorganism or enzyme.

7. An extract of brewers' spent grain (BSG) produced by the process of any one of claims 1 to 6.

8. A process for producing a food product, the process comprising the step of adding an extract of brewers' spent grain (BSG) as claimed in claim 7 to a food product or to a composition which is used in the manufacture of the food product.

9. A food product which includes an extract of brewers' spent grain (BSG) as claimed in claim 7.

10. A food product as claimed in claim 9, which is an edible product for human or animal consumption.

11. A food product as claimed in claim 10, which is an animal feed.

12. A food product as claimed in claim 9, which is a beverage.

13. A food product as claimed in claim 12, wherein the beverage is a beer or beer product.

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for the applicant